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(54) **QUANTIFICATION OF IR-A AND IR-B FOR TUMOR CLASSIFICATION**

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C12Q 1/68 (2006.01)

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CPC **C12Q 1/6886** (2013.01); **C12Q 1/6851** (2013.01); **C12Q 1/6876** (2013.01); **C12Q 2600/106** (2013.01); **C12Q 2600/112** (2013.01); **C12Q 2600/136** (2013.01); **C12Q 2600/158** (2013.01)

(58) **Field of Classification Search**

CPC **C12Q 1/6876**; **C12Q 1/6851**

USPC **435/6.12**, **6.11**; **536/241.13**, **24.33**

See application file for complete search history.

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(57) **ABSTRACT**

Without limitation, this disclosure relates to compositions and methods for detecting and quantifying the expression of insulin receptor isoform A (IR-A) and/or insulin receptor isoform B (IR-B) in a tissue sample. The disclosure also relates to methods of diagnosis and classification based at least in part upon detecting and quantifying the expression of insulin receptor isoform A (IR-A) and/or insulin B (IR-B) in a tissue sample. Methods of treating a subject based upon such a classification are among additional aspects of the disclosure presented herein.

14 Claims, 13 Drawing Sheets

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FIGURE 1A

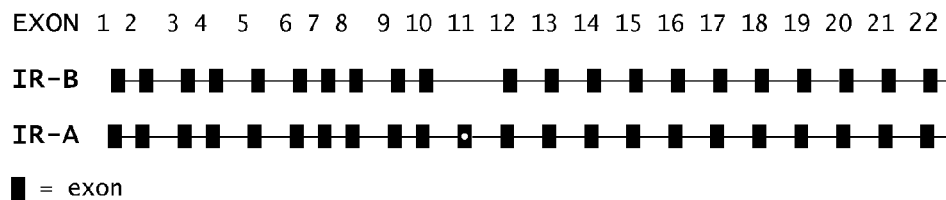


FIGURE 1B

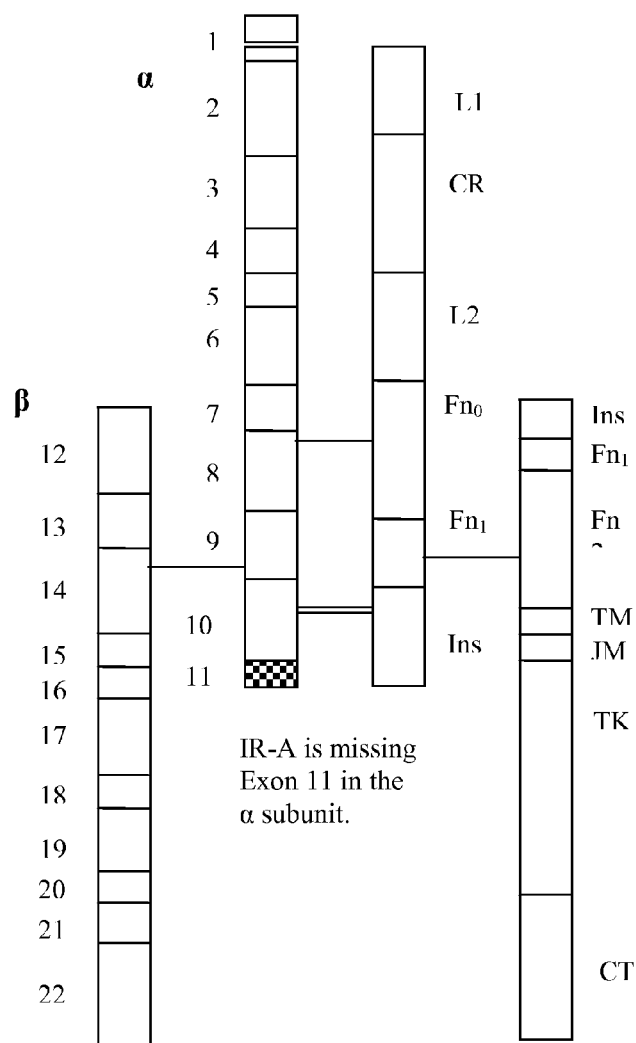


FIGURE 2

IR-A	MGTGGRGAAAAPLLVAVAALLGAAGHLYPGEVCPGMDIRNNLTRLHELENCVIEGHL	60
IR-B	MGTGGRGAAAAPLLVAVAALLGAAGHLYPGEVCPGMDIRNNLTRLHELENCVIEGHL	60
IR-A	— SIGNAL PEPTIDE — — α-Subunit —→	
IR-A	QILLMFKTRPEDFRDLSFPKLIMITDYLLLFVYGLSESLKDLFPNLTVIRGSRLFFNYAL	120
IR-B	QILLMFKTRPEDFRDLSFPKLIMITDYLLLFVYGLSESLKDLFPNLTVIRGSRLFFNYAL	120
IR-A	VIFEMVHLKELGLYNLMNITRGSVRIEKNNELCYLATIDWSRILDSVEDNYIVLNKDDNE	180
IR-B	VIFEMVHLKELGLYNLMNITRGSVRIEKNNELCYLATIDWSRILDSVEDNYIVLNKDDNE	180
IR-A	ECGDICPGTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCTAEGLCCHSECL	240
IR-B	ECGDICPGTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCTAEGLCCHSECL	240
IR-A	GNCSQPDDPTKCVACRNIFYLDGRCVETCPPPYHFQDWRCVNFSCQDLHHKCKNSRRQG	300
IR-B	GNCSQPDDPTKCVACRNIFYLDGRCVETCPPPYHFQDWRCVNFSCQDLHHKCKNSRRQG	300
IR-A	CHQYVIHNNKCIPECPSGYTMNSSNLLCTPCLGCPKVCCHLLEGEKTIDSVTSAQELRGC	360
IR-B	CHQYVIHNNKCIPECPSGYTMNSSNLLCTPCLGCPKVCCHLLEGEKTIDSVTSAQELRGC	360
IR-A	TVINGSLIINIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVLSFFRKLRLIRGETL	420
IR-B	TVINGSLIINIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVLSFFRKLRLIRGETL	420
IR-A	EIGNYSFYALDNQNLRLQLDWDSKHNLTITQGKLFHYNPKLCLSEIHKMEEVSGTKGRQE	480
IR-B	EIGNYSFYALDNQNLRLQLDWDSKHNLTITQGKLFHYNPKLCLSEIHKMEEVSGTKGRQE	480
IR-A	RNDIALKTNGDQASCENELLKFSYIRTSFDKILLRWEPYWPPDFRDLGLFMLFYKEAPYQ	540
IR-B	RNDIALKTNGDQASCENELLKFSYIRTSFDKILLRWEPYWPPDFRDLGLFMLFYKEAPYQ	540
IR-A	NVTEFDGQDAGCSNSWTVDIDPPLRSNDPKSQNHFGWLMRGLKPWTQYAI FVKTLVTFS	600
IR-B	NVTEFDGQDAGCSNSWTVDIDPPLRSNDPKSQNHFGWLMRGLKPWTQYAI FVKTLVTFS	600
IR-A	DERRTYGAKSDIIYVQTDATNPSVPLDPISVSNSSSQIILKWKPSPDNGNITHYLVFWE	660
IR-B	DERRTYGAKSDIIYVQTDATNPSVPLDPISVSNSSSQIILKWKPSPDNGNITHYLVFWE	660
IR-A	RQAEDSELFELDYLKGLKLPRTWSPPFESEDSQKHNSQSEYEDSAGECCSCPKTDSQIL	720
IR-B	RQAEDSELFELDYLKGLKLPRTWSPPFESEDSQKHNSQSEYEDSAGECCSCPKTDSQIL	720
IR-A	KELEESSFRKTFEDYLNHVVFVP-----RPSRKRRSLGDVGNVTVAVPTVAAF	768
IR-B	KELEESSFRKTFEDYLNHVVFVPRKTSSGTGAEDPRPSRKRRSLGDVGNVTVAVPTVAAF	780
IR-A	— EXON 11 — — β-Subunit —→	
IR-A	PNTSSTSVPTSPPEHRPFKEKVNKESLVISGLRHFTGYRIELQACNQDTPPEERCSVAAYV	828
IR-B	PNTSSTSVPTSPPEHRPFKEKVNKESLVISGLRHFTGYRIELQACNQDTPPEERCSVAAYV	840
IR-A	SARTMPEAKADDIVGPVTHEIFENNVVHLMWQEPKEPNGLIVLYEVSYRRYGDEELHLCV	888
IR-B	SARTMPEAKADDIVGPVTHEIFENNVVHLMWQEPKEPNGLIVLYEVSYRRYGDEELHLCV	900
IR-A	SRKHFALERGCRLRGLSPGNYSVRIRATSLAGNGSWTEPTYFYVTDYLDVPSNIAKIIIG	948
IR-B	SRKHFALERGCRLRGLSPGNYSVRIRATSLAGNGSWTEPTYFYVTDYLDVPSNIAKIIIG	960
IR-A	PLIFVFLFSVVIGSIYLFRLKRQPDGFLGPLYASSNPEYLSASDVFFCSVYVPDEWEVSR	1008
IR-B	PLIFVFLFSVVIGSIYLFRLKRQPDGFLGPLYASSNPEYLSASDVFFCSVYVPDEWEVSR	1020
IR-A	EKITLLRELQGSFGMVYEGNARDIIKGEAETRVAVKTVNESASLRERIEFLNEASVMKG	1068
IR-B	EKITLLRELQGSFGMVYEGNARDIIKGEAETRVAVKTVNESASLRERIEFLNEASVMKG	1080
IR-A	FTCHHVRLLGVVSKGQPTLVVMELMAHGDLKSYLRSRPEAENNPGRPPPTLQEMIQMA	1128
IR-B	FTCHHVRLLGVVSKGQPTLVVMELMAHGDLKSYLRSRPEAENNPGRPPPTLQEMIQMA	1140
IR-A	AEIADGMAYLNAKKFVHRDLAARNCMVAHDFTVKIGDFGMTRDIYETDYYRKGGKGLLPV	1188
IR-B	AEIADGMAYLNAKKFVHRDLAARNCMVAHDFTVKIGDFGMTRDIYETDYYRKGGKGLLPV	1200
IR-A	RWMAPESLKDGVFTTSSDMWSFGVVLWEITSLAEQPYQGLSNEQVLKFVMDGGYLDQPDN	1248
IR-B	RWMAPESLKDGVFTTSSDMWSFGVVLWEITSLAEQPYQGLSNEQVLKFVMDGGYLDQPDN	1260
IR-A	CPERVTDLMRMWCQFNPKMRPTFLEIVNLLKDDLHPSFPEVSFFHSEENKAPESSELEME	1308
IR-B	CPERVTDLMRMWCQFNPKMRPTFLEIVNLLKDDLHPSFPEVSFFHSEENKAPESSELEME	1320
IR-A	FEDMENVPLDRSSHCQREEAGGRDGGSSLGFKRSYEEHIPYTHMNGGKKNGRILTLPRSNPS	1370
IR-B	FEDMENVPLDRSSHCQREEAGGRDGGSSLGFKRSYEEHIPYTHMNGGKKNGRILTLPRSNPS	1382

FIGURE 3

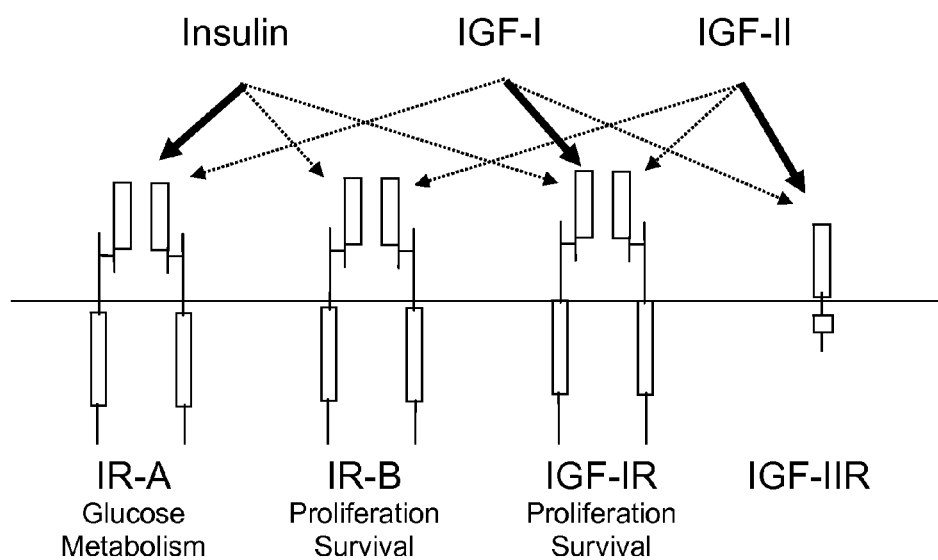


FIGURE 4

ggctgaagctgccctcgaggacctggtctccaccattcgagctctgaagattctcagaagc
Exon 10
acaaccagagtgagtatgaggattcggccggcgaatgctgctcctgtccaaagacagact

ctcagatcctgaaggagctggaggagtcctcgtttaggaagacgtttgaggattacctgc
|-----Forward-----
acaacgtgggttttcgtccccagGCCATCTCGGAAACGCAGGTCCCTTGGCGATGTTGGGA
Primer▶=====PROBE=====◀Reverse
ATGTGACGGTGGCCGTGCCCACGGTGGCAGCTTTCCCCAACACTTCCTCGACCAGCGTGC
-Primer-----|
CCACGAGTCCGGAGGAGCACAGGCCTTTTGAGAAGGTGGTGAACAAGGAGTCGCTGGTCA

TCTCCGGCTTGCGACACTTCACGGGCTATCGCATCGAGCTGCAGGCTTGCAACCAGGACA
Exon 12
CCCCTGAGGAACGGTGCAGTGTGGCAGCCTACGTCAGTGCAGGACCATGCCTGAAG

FIGURE 5

ggctgaagctgccctcgaggacctggtctccaccattcgagtctgaagattctcagaagc
Exon 10
acaaccagagtgagtatgaggattcggccggcgaatgctgctcctgtccaaagacagact

ctcagatcctgaaggagctggaggagtcctcgtttaggaagacgtttgaggattacctgc
|—Forward-Primer
acaacgtgggttttcgtccccagGCCATCTCGGAAACGCAGGTCCCTTGGCGATGTTGGGA
—————→ ==PROBE== ←Reverse-Primer|
ATGTGACGGTGGCCGTGCCACGGTGGCAGCTTTCCCCAACACTTCCTCGACCAGCGTGC

CCACGAGTCCGGAGGAGCACAGGCCTTTTGAGAAGGTGGTGAACAAGGAGTCGCTGGTCA

TCTCCGGCTTGCGACACTTCACGGGCTATCGCATCGAGCTGCAGGCTTGCAACCAGGACA
Exon 12
CCCCTGAGGAACGGTGCAGTGTGGCAGCCTACGTCAGTGCGAGGACCATGCCTGAAG

FIGURE 6

ggctgaagctgccctcgaggacctgggtctccaccattcgagtctgaagattctcagaagc
Exon 10
acaaccagagtgagtatgaggattcggccggcgcaatgctgctcctgtccaaagacagact

ctcagatcctgaaggagctggaggagtcctcggttaggaagacgtttgaggattacctgc

acaacgtgggttttcgtccccagAAAAACCTCTTCAGGCACTGGTGCCGAGGACCCTAGgcc
Exon 11
|—Forward Primer—→ ==Probe IRB4==
 ==Probe IRB3==
 ==Probe IRB5==
atctcgaaacgcaggtcccttggcgatggtgggaatgtgacggtggccgtgccacaggtg
←Reverse Primer—|
gcagctttccccaacacttcctcgaccagcgtgcccacgagtcgaggaggcacaggcctt

ttgagaaggtggtgaacaaggagtcgctgggtcatctccggcttgcgacacttcacgggcta

tcgcatcgagctgcaggcttgcaaccaggacacccctgaggaacggtgcagtggtggcagcc
Exon 12
tacgtcagtcgcaggaccatgcctgaag

FIGURE 7

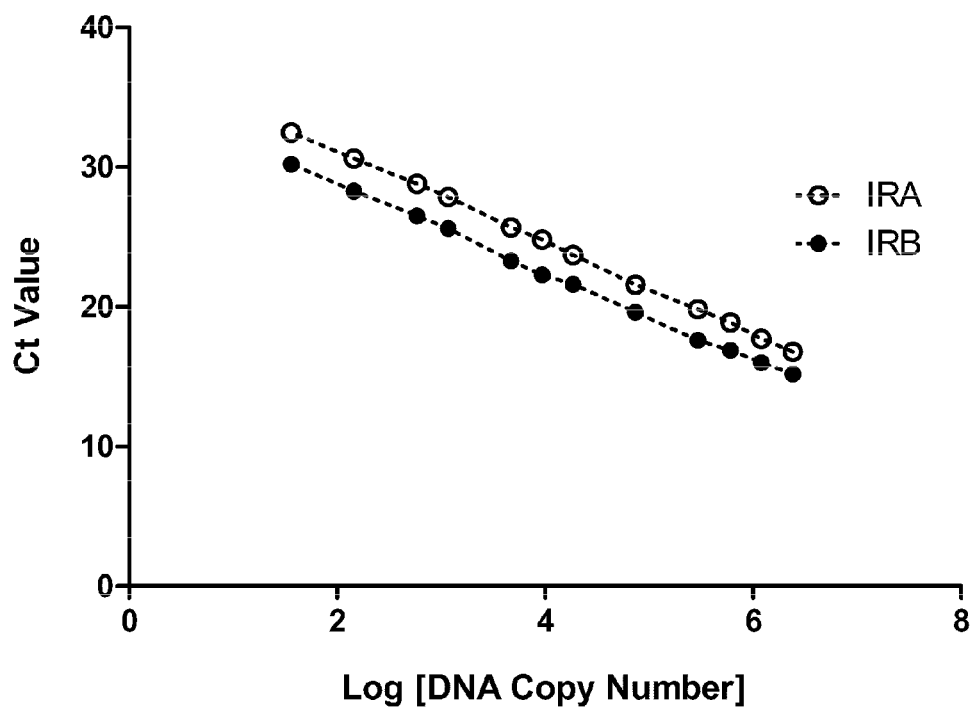


FIGURE 8

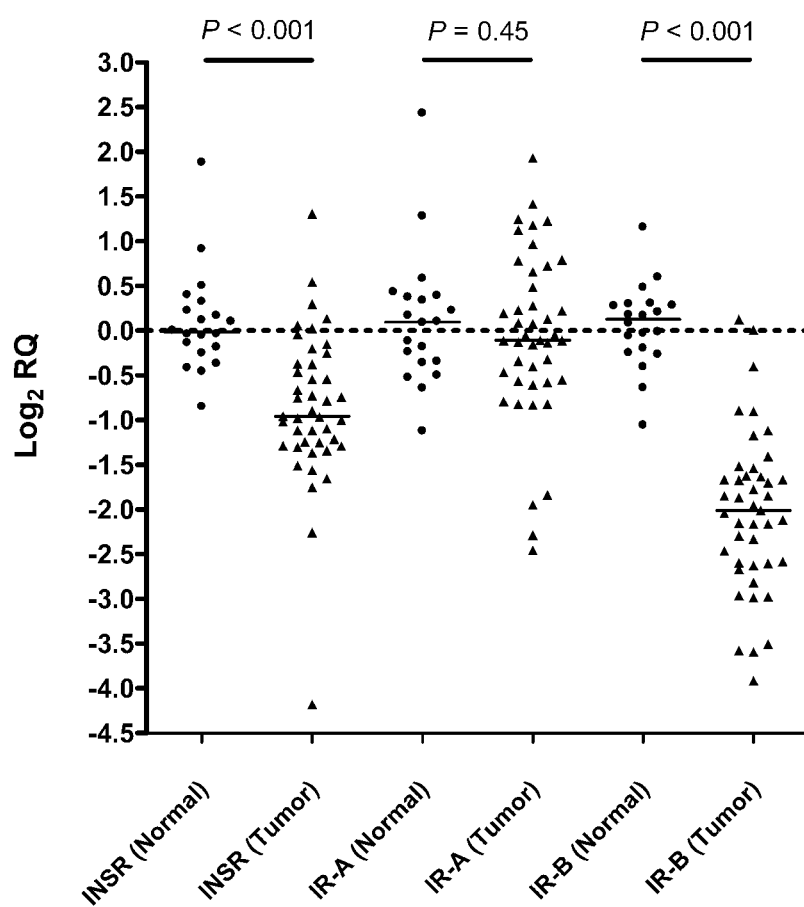


FIGURE 9

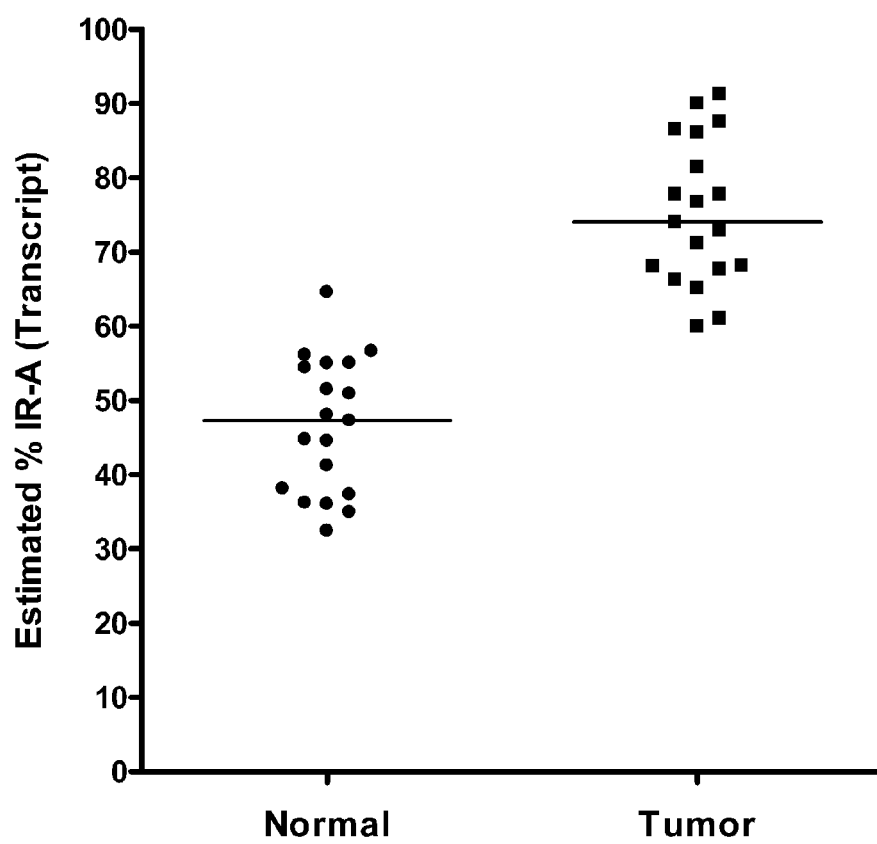


FIGURE 10

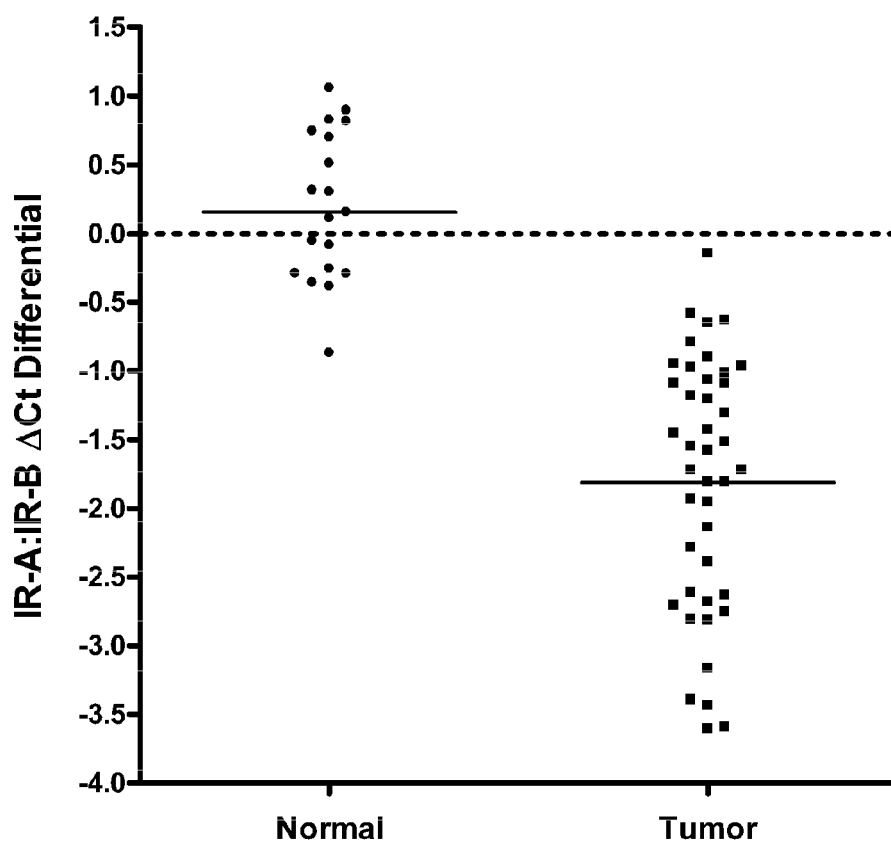


FIGURE 11

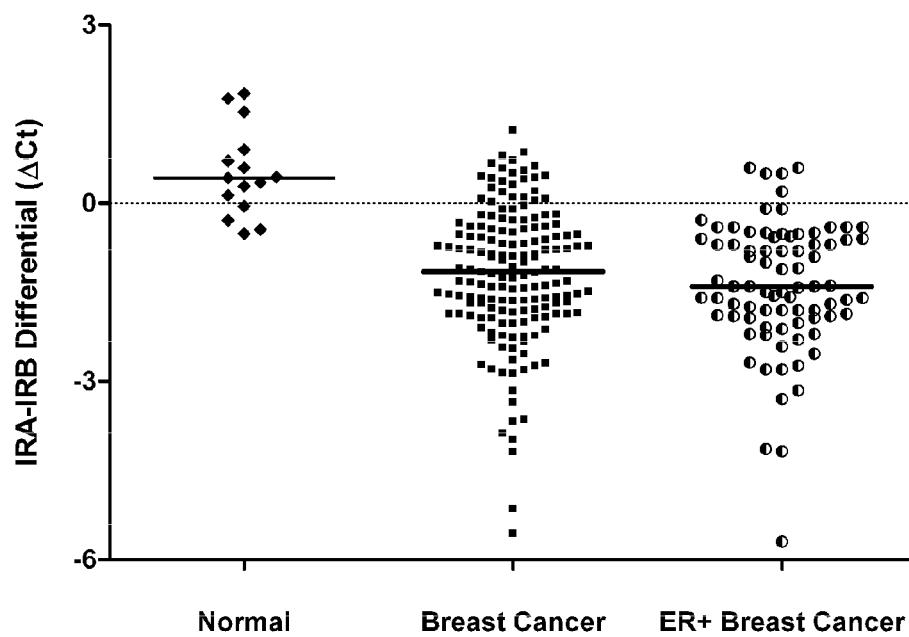


FIGURE 12

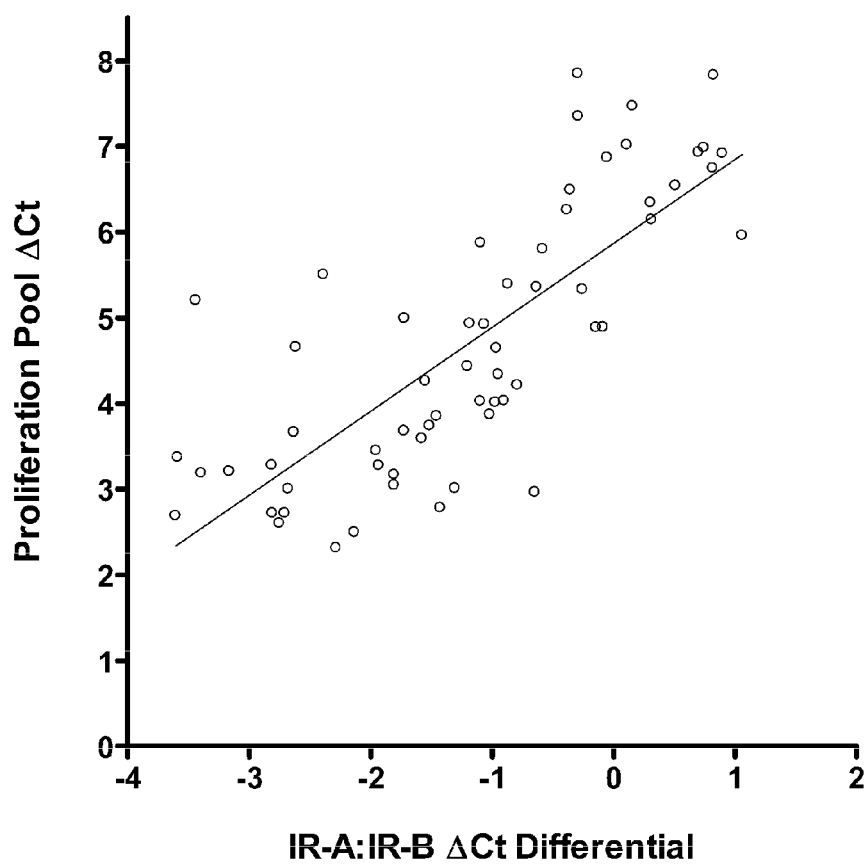
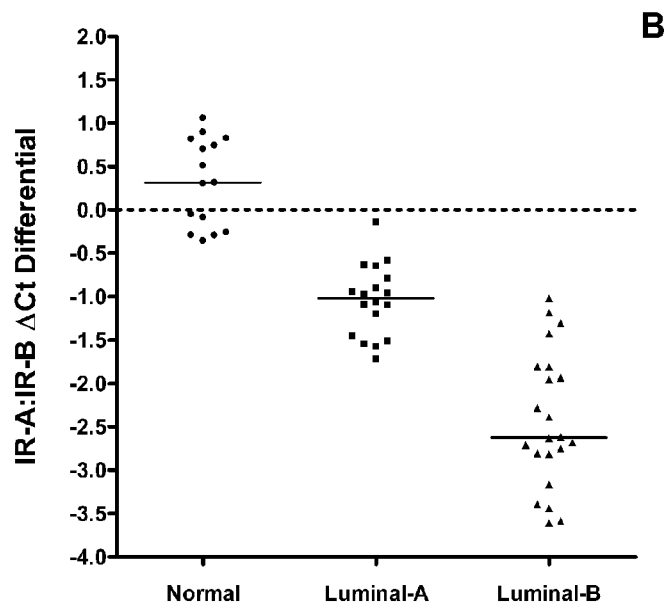
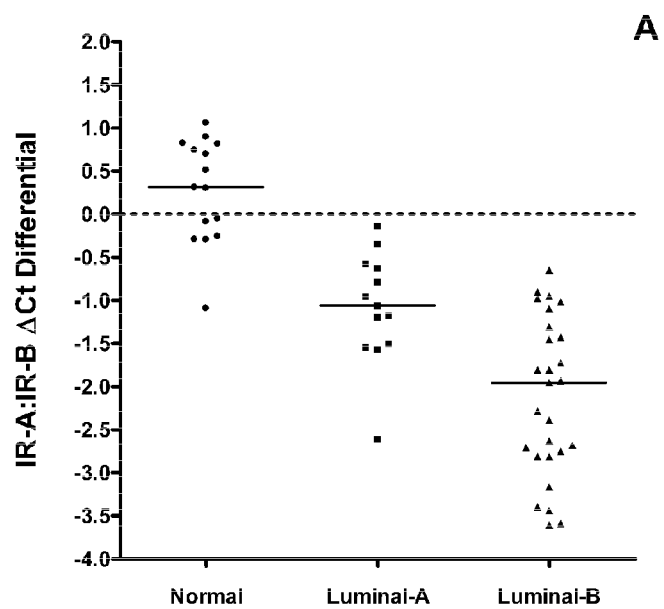


FIGURE 13



1

**QUANTIFICATION OF IR-A AND IR-B FOR
TUMOR CLASSIFICATION****CROSS-REFERENCE TO RELATED
APPLICATIONS**

This application is a U.S. National Stage application of International Application No. PCT/US2010/052173, filed on Oct. 11, 2010, said International Application No. PCT/US2010/052173 claims benefit under 35 U.S.C. §119(e) of the U.S. Provisional Application No. 61/250,780, filed Oct. 12, 2009. Each of the above listed applications is incorporated by reference herein in its entirety for all purposes.

SEQUENCE LISTING

This application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is incorporated by reference in its entirety. Said ASCII copy, created on Jun. 27, 2013, is named IGF-400US1_SL.txt and is 49,396 bytes in size.

BACKGROUND**1. Field of the Disclosure**

Without limitation, this disclosure relates to compositions and methods for detecting and quantifying the expression of insulin receptor isoform A (IR-A) and/or insulin receptor isoform B (IR-B) in a sample, such as a tissue sample. The disclosure also relates to methods of diagnosis and classification based at least in part upon detecting and quantifying the expression of insulin receptor isoform A (IR-A) and/or insulin receptor isoform B (IR-B) in a sample, such as a tissue sample. Methods of treating a subject based upon such a classification are among additional aspects of the disclosure presented herein.

2. Description of the Related Art

The insulin receptor (INSR) is a transmembrane tyrosine kinase receptor implicated in regulation of energy metabolism. The INSR comprises two subunits, α and β , expressed from a single INSR gene. Two isoforms, designated IR-A and IR-B, are the result of alternative mRNA splicing. IR-A is generated by an alternative splicing of the mRNA transcribed from the INSR gene to omit exon 11, which is included in mRNA of IR-B. (FIGS. 1A-1B). Thus, IR-A differs from IR-B because it lacks a stretch of amino acid residues at the carboxy terminus of the INSR α -subunit. IR-A and IR-B are more than 99% identical (FIG. 2). While the expression profiles of the two isoforms are different, the isoforms are coexpressed in cells. The relative abundance of IR-A and IR-B is regulated by development stage- and tissue-specific factors.

For example, IR-A is predominantly expressed in fetal and cancer cells, whereas IR-B is predominantly expressed in differentiated insulin target cells. IR-A exhibits high affinity for insulin, intermediate affinity for IGF-II, and low affinity for IGF-I. IGF-II binds to IGF type I receptor (IGF-IR) and to IR-A with similar affinities. IR-B is a highly specific receptor for insulin.

Currently methods of determining IR-A and IR-B levels in a patient sample are hampered by the lack of an efficient and accurate means of detection. To date, the only available method to specifically measure IR-A expression has been described by Sciacca, et al (Oncogene 21(54):8240-50; 2002 Nov. 28). This method is based on PCR and gel separation, followed by qualitative measurement of the resulting bands.

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This method is very labor intensive and is not quantitatively accurate, which limits its use in a high throughput or clinical setting.

SUMMARY

There is a need for improved diagnostic tests that can predict a patients' response to a drug based on a determination of whether the patient's tissues express a molecular target of the drug. There is need for a method to accurately detect and/or quantify the expression of IR-A and IR-B. The ability to be able to detect and quantify the expression of each isoform in a patient sample is useful to provide a patient with a personalized treatment regime. Such personalized treatment regimes offer the potential to maximize therapeutic benefit to the patient, while minimizing, for example, side effects that may be associated with alternative and less effective treatment regimes.

Disclosed herein are compositions and methods that are useful for detecting and/or quantifying whether a sample of biological material contains cells that express IR-A and/or IR-B, including human IR-A and/or IR-B, and kits for use in such a method. The methods are based, in part, on the finding that particular oligonucleotides can be used to measure the expression of IR-A and/or IR-B in tissue samples. These oligonucleotides can be used to quickly and quantitatively distinguish the level of IR-A and/or IR-B expression in a sample such that the methods can be used in high throughput and clinical settings. These compositions and methods can be useful in methods to classify tissue samples, for example tumor tissue samples. The results of such methods may be used as a factor in classifying a tumor or cancer patient as an indicator of how a patient will respond to drugs such as antagonists or agonists of INSR, IGF1R, or IGF. These methods and the resulting classifications can be used in methods of selecting candidates for treatment and methods of treating cancer patients.

Useful oligonucleotides can comprise synthetic nucleic acids that can be used to selectively amplify IR-A and/or IR-B. Such useful oligonucleotides can comprise a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides, wherein the synthetic nucleic acid sequence comprises at least 10-20 consecutive nucleotides of any one of the following sequences: (i) SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions; (ii) SEQ ID NO: 4, a sequence complementary thereto (SEQ ID NO:21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions, or a variant thereof; (iii) SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions; or (iv) SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions is disclosed. The synthetic nucleic acid sequence may consist essentially of any one of the following nucleic acid sequences: (i) SEQ ID NO: 3 (TGAGGATTACCTGCACAACG), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions, or a variant thereof; (ii) SEQ ID NO: 4 (GATGTTGGGA ATGTGACGGT), a sequence complementary thereto (SEQ ID NO:21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions; (iii) SEQ ID NO: 5 (TTGAGGATTACCTGCACAACGT), a sequence complementary thereto, or a sequence that is

capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions, or a variant thereof; or (iv) SEQ ID NO: 6 (AAACGCAGGTCCTTGGC), a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions, or a variant thereof.

A useful synthetic nucleic acid sequence may SEQ ID NO: 7, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions, or a variant thereof or SEQ ID NO: 8, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions, or a variant thereof. Another useful synthetic nucleic acid sequence may consist essentially of SEQ ID NO: 7, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions or a variant thereof, or SEQ ID NO: 8, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions or a variant thereof. A composition comprising any of the synthetic nucleic acid sequences described above can also be useful.

Also disclosed herein is a primer set that is useful for determining the presence or absence of a target human IR-A nucleic acid sequence in a biological sample, wherein the primer set comprises at least one synthetic nucleic acid sequence that may be chosen from among a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of at least one of the following: (a) the last 50 bases of exon 10 of the INSR gene (SEQ ID NO: 1) or a complementary nucleic acid sequence thereof; and (b) the first 60 bases of exon 12 of the INSR gene (SEQ ID NO: 2), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions. The synthetic nucleic acid sequence may have a nucleotide sequence that may be chosen from among SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions; SEQ ID NO: 4, a sequence complementary thereto (SEQ ID NO:21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions; SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions; and SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions.

Also disclosed herein is a method for determining the presence or absence of a target IR-A nucleic acid sequence in a biological sample, comprising the steps of: (a) contacting a biological sample with a primer set such as described above under conditions suitable for polymerase-based amplification; and (b) detecting and/or quantifying amplified target IR-A nucleic acid sequence. As examples, a biological sample can be a tissue sample such as a tumor sample or a sample comprising nucleic acids derived from a tissue or cell sample.

A primer set can include SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions, and SEQ ID NO: 4, a sequence complementary thereto (SEQ ID NO:21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions. In another example, the primer set can include SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or

its complement, under stringent conditions, and SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions. In one example, the polymerase-based amplification is quantitative polymerase chain reaction (q-PCR).

A primer and probe set can include: SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions, or a variant thereof; SEQ ID NO: 4 (SEQ ID NO:21), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions, or a variant thereof; and SEQ ID NO: 7, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions, or a variant thereof, wherein said sequence may also comprise a detectable label and may further comprise a quencher. In another example, a primer and probe set can include: SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions, or a variant thereof; SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions, or a variant thereof; and SEQ ID NO: 8, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions, or a variant thereof, wherein said sequence may also include a detectable label and may further include a quencher. Typically, the amplified product is less than 100 bases.

A useful synthetic nucleic acid sequence can comprise 10-30 consecutive nucleotides, wherein the synthetic nucleic acid sequence comprises at least 10-20 consecutive nucleotides of any one of the following sequences: SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions, or a variant thereof; or SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions, or a variant thereof. In one example, the nucleic acid sequence consists essentially of: SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions, or a variant thereof; or SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions, or a variant thereof; SEQ ID NO: 13, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:13, or its complement, under stringent conditions, or a variant thereof; SEQ ID NO: 14, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:14, or its complement, under stringent conditions, or a variant thereof or SEQ ID NO: 15, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:15, or its complement, under stringent conditions, or a variant thereof. A composition comprising any one or more of the synthetic nucleic acid sequences above may also be useful.

A primer set for determining the presence or absence of IR-B in a biological sample can comprise a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of at least one of the following: (a) the last 50 bases of exon 10 (SEQ ID NO: 1) of the INSR gene, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:1, or its complement, under stringent conditions,

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and the bases of exon 11 (SEQ ID NO: 9) of the INSR gene, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:9, or its complement, under stringent conditions or a sequence complementary thereto, or which bridges exon 10 and exon 11 of the INSR gene, a sequence complementary thereto, or a sequence that is capable of hybridizing thereto, or its complement, under stringent conditions; and (b) the first 50 bases of exon 12 of the INSR gene (SEQ ID NO: 10), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:10, or its complement, under stringent conditions or a sequence complementary thereto. A primer set can include at least one synthetic nucleic acid sequence has a nucleotide sequence that may be chosen from among SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; and SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions.

A method for determining the presence or absence of a target IR-B nucleic acid sequence in a biological sample may comprise the steps of: (c) contacting a biological sample with the primer set of above under conditions suitable for polymerase-based amplification; and (d) detecting and/or quantifying amplified target IR-B nucleic acid sequence. As an example, the biological sample may be a sample comprising nucleic acid from a tissue sample such as a tumor sample. In another example, the polymerase-based amplification may be by a quantitative polymerase chain reaction. A primer and probe set may include: SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and SEQ ID NO: 13, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:13, or its complement, under stringent conditions which may also include a detectable label and may further include a quencher. In another example, a primer and probe set may include: SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and SEQ ID NO: 14, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:14, or its complement, under stringent conditions.

In another example, a primer set can include: SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and further comprise SEQ ID NO: 15, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:15, or its complement, under stringent conditions which may also include a detectable label and may further include a quencher. Typically the amplified product is less than 100 bases.

A method for determining the presence or absence of a target IR-A and IR-B nucleic acid in a biological sample may comprise the steps of: contacting a biological sample with an IR-A primer set as described above under conditions suitable for polymerase-based amplification; contacting a biological

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sample with an IR-B primer set as described above under conditions suitable for amplification by polymerase reaction; and detecting and/or quantifying amplified target IR-A and IR-B nucleic acid sequence. The method may further comprise calculating a relative expression level of IR-A and IR-B.

A kit for determining the presence or absence of IR-A in a biological sample may comprise at least one synthetic nucleic acid sequence described above and instructions for carrying out one or more of the methods described herein. In such a kit, the at least one synthetic nucleic acid sequence may have a nucleotide sequence chosen from among the IR-A primers and probes disclosed herein, or any sets thereof. A kit can also include suitable PCR reagents; and optionally, a positive and/or negative control for determining the presence or absence of IR-A.

A kit for determining the presence or absence of IR-B in a biological sample can comprise at least one synthetic nucleic acid as described above and instructions for performing one or more methods described herein. A synthetic nucleic acid in such a kit may have a nucleotide sequence chosen from among the IR-B nucleic acids. The kit may also include suitable PCR reagents; and optionally, a positive and/or negative control for determining the presence or absence of IR-B.

A method for selecting a patient responsive to an IGF1/II ligand, INSR, or IGFR1 receptor antagonist or agonist can comprise obtaining a biological sample from a subject having or suspected of having cancer; and detecting and/or quantifying the presence of IR-A in the sample. The presence or absence of IR-A is an indication as to whether an IGF1/II ligand, INSR, or IGFR1 receptor antagonist or agonists should be administered to the subject. Thus, a method of treating a patient may comprise detecting and/or quantifying the presence of IR-A in a patient that has been obtained from a patient and administering an IGF1/II ligand, INSR, or IGFR1 receptor antagonist or antagonist to the patient. As an example, the IGF1 and II ligand, INSR, or IGFR1 receptor antagonist may be an antibody.

A tumor may be classified according to the expression of IR-A and or IR-B, or by the relative amounts of IR-A and IR-B. Classifying tumors in this way provides an ability to identify tumors that have overexpressed IR-A and/or IR-B or that have an altered amount of IR-A relative to IR-B or vice versa. A method of classifying a tumor can comprise quantifying the expression of IR-A and/or IR-B or the amount of IR-A relative to IR-B or vice versa in a sample of tumor tissue and assigning a classification based upon the quantification. A method of treating a patient having a tumor can comprise classifying the tumor by quantifying the expression of IR-A and/or IR-B or the amount of IR-A relative to IR-B or vice versa in a sample of tumor tissue; assigning a classification based upon that quantification of the expression of IR-A and/or IR-B or the amount of IR-A relative to IR-B or vice versa; and administering an IGF1/II ligand or IGFR1 receptor antagonist or agonist to the patient in accordance with the classification.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B illustrate IR-A and IR-B. FIG. 1A shows the genomic structure of IR-A and IR-B. FIG. 1B presents a schematic of the α and β subunits of an INSR homodimer with exon locations illustrated on the left and functional domain locations illustrated on the right.

FIG. 2 shows a sequence alignment of human IR-A (SEQ ID NO: 32) and IR-B (SEQ ID NO: 33) demonstrating that the sequences are identical except for the omission of exon 11 from IR-A.

FIG. 3 shows a schematic of IGF-1R/IR receptors illustrating strong and weak ligand interactions.

FIG. 4 shows a nucleotide sequence encoding a portion of IR-A (SEQ ID NO: 34) and a schematic of primer and probe locations for an example IR-A assay.

FIG. 5 shows a nucleotide sequence encoding a portion of IR-A (SEQ ID NO: 34) and a schematic of primer and probe locations for an example IR-A assay.

FIG. 6 shows a nucleotide sequence encoding a portion of IR-B (SEQ ID NO: 35) and a schematic of primer and probe locations for an example IR-B assay.

FIG. 7 shows results of IR-A or IR-B qRT-PCR assays with a serial dilution of approximately 10^7 to 10 copies of plasmid DNA of either IR-A, IR-B, or an empty vector control. The Y axis represents cycle-threshold (Ct) values and the X axis represents log DNA copy number. The slope and correlation coefficient (r^2 value) of the standard dilution curve for the IR-A assay are -3.259 and 0.9992, respectively. The slope and correlation coefficient (r^2 value) of the standard dilution curve for the IR-B assay are -3.155 and 0.9989, respectively.

FIG. 8 shows relative mRNA expression levels of insulin receptor and its isoforms in primary breast cancer compared to normal breast tissues. TaqMan gene expression assay determined relative quantity (RQ) differentials (\log_2 -base scale) of INSR (total), IR-A, and IR-B between normal (n=19) and tumor (n=42) breast tissue samples. Average normal Δ Ct values were used for calculation of fold change differentials for each sample. A two-tailed Welch's t-test analysis identified a significant difference between normal and tumor samples for both INSR and IR-B ($P<0.001$), whereas no difference was observed for IR-A ($P=0.45$). Bars represent the median \log_2 RQ value within a particular gene target and tissue-type combination.

FIG. 9 shows the proportion of IR-A expression in matched normal breast and breast cancer specimens. Within sample proportion of insulin receptor isoform-A (IR-A) relative to total insulin receptor composition (i.e., IR-A+IR-B) as determined by $2^{(-\Delta\Delta C_t)}$ calculation. A paired sample t-test analysis indicated that a significant difference exists for calculated IR-A proportion between matched normal and tumor samples ($P<0.001$). Black bars represent the median IR-A proportion (%) within the normal (46.60 ± 4.74 , mean $\pm 95\%$ CI) and tumor (75.24 ± 5.03 , mean $\pm 95\%$ CI) tissue.

FIG. 10 shows increased IR-A:IR-B ratio in primary breast cancer. Calculated Δ Ct differentials of insulin receptor isoforms IR-A and IR-B in normal (n=19) and tumor (n=42) breast samples. Δ Ct differentials (IR-A Δ Ct-IR-B Δ Ct) values were calculated for all samples utilizing the within-sample reference gene panel (average Ct) for normalization purposes. A two-tailed Welch's t-test analysis identified a significant difference between normal and tumor samples in relation to observed IR-A:IR-B Δ Ct differential ($P<0.001$). Black bars represent the median IR-A:IR-B Δ Ct differential within a particular tissue-type.

FIG. 11 shows an increased IR-A:IR-B ratio in breast cancer samples from qPCR cDNA array. Calculated Δ Ct differentials of insulin receptor isoforms IR-A and IR-B (Y axis) in normal (n=15; samples from 10 independent donors), breast cancer cDNA panels (n=165), and breast tumors from these cDNA panels with >2 -fold estrogen receptor (ER) over-expression (n=83). Δ Ct differentials (IR-A Δ Ct-IR-B Δ Ct) values were calculated for all samples utilizing the within-sample reference gene panel (average Ct) for normalization purposes. A two-tailed Welch's t-test analysis identified a significant difference between normal and tumor samples in

relation to observed IR-A:IR-B Δ Ct differential ($P<0.0001$). Bars represent the mean IR-A:IR-B Δ Ct differential within a particular tissue-type.

FIG. 12 shows a correlation of IR-A:IR-B Δ Ct differential with the expression of proliferation genes. Linear regression analysis of the relationship between calculated IR-A:IR-B Δ Ct differential (Y axis) and a pooled panel of proliferation markers (AURKA, BIRC5, CCNB1, KI67, and MYBL2) (X axis). Proliferation panel summary values were calculated by taking the mean Δ Ct across all markers for a particular sample. Summary results for both normal and tumor samples are presented. The linear regression analysis results suggest a positive correlation between the two summary values (adjusted $R^2=0.595$).

FIGS. 13 (A and B) show IR-A:IR-B Δ Ct differential in subtypes of ER+breast cancer. Scatter plot representation of calculated IR-A:IR-B Δ Ct differentials with regard to sample subtype (normal, luminal-A, or luminal-B) classification determined by a shrunken centroid classifier-based methodology (A) and a two-sample Welch's t-test analysis (B). All subtype pair-wise comparisons display a significant difference (two-sample t-test, $p<0.001$). Bars represent the median IR-A:IR-B Δ Ct differential within a particular sample subtype.

DETAILED DESCRIPTION

The following primer and probe sequences are referred to herein.

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                                SEQ ID NO: 1
(TTTAGGAAGA CGTTTGAGGA TTACCTGCAC AACGTGGTTT
TCGTCCCCAG) INSR Exon 10 C-term

                                SEQ ID NO: 2
(GCCATCTCGG AAACGCAGGT CCCTTGCGA TGTTGGGAAT
GTGACGGTGG CCGTGCCCAC) INSR Exon 12 N-term

                                SEQ ID NO: 3
(TGAGGATTACCTGCACAACG) IR-A Primer

                                SEQ ID NO: 4
(GATGTTGGGA ATGTGACGGT) IR-A Primer (reverse
complement SEQ ID NO: 21)

                                SEQ ID NO: 5
(TTGAGGATTA CCTGCACAACGT) IR-A Primer

                                SEQ ID NO: 6
(AAACGCAGGTCCCTTGGC) IR-A Primer (reverse
complement SEQ ID NO: 22)

                                SEQ ID NO: 7
(TCCCCAGGCCATCT) IR-A Probe

                                SEQ ID NO: 8
(TTTTCGTCCCCAGGCCA) IR-A Probe

                                SEQ ID NO: 9
(AAAAACCTCT TCAGGCACTG GTGCCAGGA CCCTAG) INSR
Exon 11

                                SEQ ID NO: 10
(GCCATCTCGG AAACGCAGGT CCCTTGCGA TGTTGGGAAT
GTGACGGTGG) INSR Exon 12 N-term

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-continued

(CGTCCCCAGAAAACCTCTTC) IR-B Primer SEQ ID NO: 11
 (GGACCTGCGTTTCCGAGAT) IR-B Primer SEQ ID NO: 12
 (ACTGGTGCCGAGGAC) IR-B Specific Probe SEQ ID NO: 13
 (CCGAGGACCTAGGC) IR-B Specific Probe SEQ ID NO: 14
 (TGCCGAGGACCTAG) IR-B Specific Probe SEQ ID NO: 15

Further primer and probe sequences include those in Tables 3 and 4. The skilled artisan will be able to select primers pairs from the primers disclosed herein, that are capable of amplifying nucleic acid sequences in a PCR reaction (e.g., anneal to opposite strands and prime DNA synthesis in the proper direction). The skilled artisan will understand that the complement of such primers can be used, for example, as negative controls.

These nucleic acid sequences and related sequences described herein can be used in assays to detect and quantify expression of human IR-A and human IR-B in a sample despite the high sequence identity between IR-A and IR-B. Thus, for the first time a quick and sensitive method to determine expression of IR-A and/or IR-B in sample has been discovered. The methods described using these sequences are quantitatively accurate, allowing them to be used in high throughput and clinical settings.

A synthetic nucleic acid sequence or oligonucleotide that can be used to identify the expression of IR-A and/or IR-B in a test sample may be DNA, RNA, chimeric mixtures or derivatives or modified versions thereof that can be modified at the base moiety, sugar moiety or backbone and may include other appending groups, labels or quenchers, so long as they are still capable of functioning in the desired reaction. The synthetic nucleic acid sequences may comprise at least one modified phosphate backbone—such as phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or an analogue thereof.

A. IR-A Nucleic Acid Sequences

Suitable IR-A synthetic nucleic acid sequences include those appearing in Tables 3 and 4.

Synthetic nucleic acids comprising IR-A nucleic acid sequences that occur in the last 50, 45, 40, 35, 30, 25 or 20 bases of exon 10 of the INSR gene (SEQ ID NO: 1) or a sequence that is capable of hybridizing to SEQ ID NO:1, or its complement, under stringent conditions, and the first 60, 55, 50, 45, 40, 35, 30, 25 or 20 bases of exon 12 of the INSR gene (SEQ ID NO: 2) or a sequence that is capable of hybridizing to SEQ ID NO:2, or its complement, under stringent conditions can be used in polymerase-based amplification and detection such as quantitative polymerase chain reaction (qPCR) (also known as real-time PCR or kinetic PCR) to determine the level of expression of IR-A in a sample.

A synthetic nucleic acid comprising an IR-A sequence can include 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 consecutive nucleotides, wherein the synthetic nucleic acid sequence comprises at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, consecutive nucleotides of any one of the following sequences:

- (i) SEQ ID NO: 3 (TGAGGATTAC CTGCACAACG), a sequence complementary thereto, or a sequence that is

capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions;

- (ii) SEQ ID NO: 4 (GATGTTGGGA ATGTGACGGT), a sequence complementary thereto (SEQ ID NO:21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions;
- (iii) SEQ ID NO: 5 (TTGAGGATTAC CCTGCACAACGT), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions; or
- (iv) SEQ ID NO: 6 (AAACGCAGGT CCCTTGGC), a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions.

In one example, a primer set for determining the presence or absence of a target IR-A nucleic acid sequence in a biological sample can comprise at least one synthetic nucleic acid sequence that may be chosen from among a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of at least one of the following: (a) the last 50, 45, 40, 35, 30, 25 or 20 bases of exon 10 of the INSR gene (SEQ ID NO: 1), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:1, or its complement, under stringent conditions; and (b) the first 60, 55, 50, 45, 40, 35, 30, 25 or 20 bases of exon 12 of the INSR gene (SEQ ID NO: 2), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:2, or its complement, under stringent conditions. The primer set can include (i) SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions and SEQ ID NO: 4, a sequence complementary thereto (SEQ ID NO:21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions or (ii) SEQ ID NO: 5, or complementary sequence thereof; and SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO:22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions. Typically the primer set is used in PCR to produce a product between about 50-150 bp. Probes may comprise SEQ ID NO: 7 or 8, or a sequence that is capable of hybridizing to either SEQ ID NO:7 or 8, or its complement, under stringent conditions, and may also comprise an appropriate label and quencher combination.

As an example, a primer set useful in a quantitative polymerase chain reaction (qPCR) includes:

SEQ ID NO: 3, or a nucleic acid sequence that is complementary thereto;

SEQ ID NO: 4, or a nucleic acid sequence that is complementary thereto (SEQ ID NO: 21); and an additional synthetic nucleic acid or probe having a detectable label that may also include a quencher. In one example, the additional nucleic acid is a single stranded nucleic acid sequence of between 10-50 nucleotides and is designed to bind specifically to the DNA sequence of an IR-A cDNA or its complement between the two PCR primers of SEQ ID NO: 3 and 4. The additional nucleic acid typically has a fluorescent reporter or fluorophore such as 6-carboxyfluorescein (FAM) or tetrachlorofluorescein (TET) and a quencher such as tetramethylrhodamine (TAMRA) covalently attached at its 5' and 3' ends. In this example, only specific PCR products generate a fluorescent signal. In this example, the additional nucleic acid can be 10-50 bases and include at least 5-14 bases of SEQ ID NO: 7 or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions.

In another example, the primer set comprises:

SEQ ID NO: 5, or a nucleic acid sequence that is complementary thereto;

SEQ ID NO: 6, or a nucleic acid sequence that is complementary thereto (SEQ ID NO: 22); and an additional nucleic acid having a detectable label that may also include a quencher. The additional nucleic acid can be a single stranded nucleic acid sequence of between 10-32 nucleotides and is designed to bind only the DNA sequence of an IR-A cDNA or its complement between the two PCR primers of SEQ ID NO: 5 and 6. In this example, the additional nucleic acid can be 10-32 bases and include at least 5-14 bases of SEQ ID NO: 8 or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions.

B. IR-B Nucleic Acid Sequences

Suitable IR-B synthetic nucleic acid sequences include those appearing in Tables 3 and 4.

IR-B nucleic acid sequences that occur in the (a) last 50, 45, 40, 35, 30, 25 or 20 bases of exon 10 (SEQ ID NO: 1) or its complement or a sequence that is capable of hybridizing to SEQ ID NO:1, or its complement, under stringent conditions and exon 11 of the INSR gene (SEQ ID NO: 9) or a sequence complementary thereto or a sequence that is capable of hybridizing to SEQ ID NO:9, or its complement, under stringent conditions; and (b) the first 50, 45, 40, 35, 30, 25 or 20 bases of exon 12 of the INSR gene (SEQ ID NO: 2), a sequence complementary thereto or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions can be used in the methods disclosed herein to determine the level of expression of IR-B, particularly PCR based methods.

In one example, synthetic nucleic acids comprising IR-B sequences can include 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 consecutive nucleotides, wherein the synthetic nucleic acid sequence comprises at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, consecutive nucleotides of any one of the following sequences:

(i) SEQ ID NO: 11 or a sequence complementary thereto or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; or

(ii) SEQ ID NO: 12 or a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions.

In another example, a primer set for determining the presence or absence of a target IR-B nucleic acid sequence in a biological sample may comprise at least one synthetic nucleic acid sequence that may be chosen from among a synthetic nucleic acid sequence comprising 10-27 consecutive nucleotides of at least one of the following: (a) 50, 45, 40, 35, 30, 25 or 20 bases in the last 50 bases of exon 10 (SEQ ID NO:1) and exon 11 of the INSR gene (SEQ ID NO: 9) or a sequence complementary thereto; and (b) the first 50, 45, 40, 35, 30, 25 or 20 bases of exon 12 of the INSR gene (SEQ ID NO: 10) or a sequence complementary thereto. The primer set can include a nucleotide sequence that may be chosen from among SEQ ID NO: 11, or a synthetic nucleic acid sequence complementary thereto; and SEQ ID NO: 12, or a synthetic nucleic acid sequence complementary thereto.

When the primer set is used in a PCR such as qPCR, the primer set can include SEQ ID NO: 11, or a synthetic nucleic

acid sequence that is complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; SEQ ID NO: 12, or a synthetic nucleic acid sequence that is complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and an additional nucleic acid comprising a detectable label that may also include a quencher. The additional nucleic acid can be a single stranded nucleic acid sequence of between 10-27 nucleotides and is designed to bind specifically to the sequence of an IR-B cDNA or its complement between the two PCR primers of SEQ ID NO: 11 and 12 or a sequence that is capable of hybridizing to that region, or its complement, under stringent conditions. As an example, the additional nucleic acid can be 10-27 bases and include at least 5-14 bases of SEQ ID NO: 13, or its complement, or a sequence that is capable of hybridizing to SEQ ID NO: 13, or its complement, under stringent conditions. The additional nucleic acid can be 10-30 bases and include at least 5-14 bases of SEQ ID NO: 14, or its complement, or a sequence that is capable of hybridizing to SEQ ID NO: 14, or its complement, under stringent conditions. In yet another example, the additional nucleic acid can be 10-30 bases and include at least 5-14 bases of SEQ ID NO: 15, or its complement, or a sequence that is capable of hybridizing to SEQ ID NO:15, or its complement, under stringent conditions. The additional nucleic acid typically has a fluorescent reporter or fluorophore such as 6-carboxyfluorescein (FAM) and tetrachlorofluorescein (TET) and a quencher such as tetramethylrhodamine (TAMRA) covalently attached at its 5' and 3' ends.

Useful synthetic nucleic acid sequences also include variants of the sequences disclosed above or sequences that are substantially similar to the nucleic acids disclosed herein. Variants include sequences that are altered by one or more bases, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 but can still anneal to the specific locations on the IR-A target sequence of interest. The term "substantially similar" when used in relation to annealing or hybridization, means that a synthetic nucleic acid sequence, such as a primer, should be sufficiently complementary to hybridize or anneal to its respective nucleic acid under stringent conditions. The synthetic nucleic acid sequence need not reflect the exact sequence of its respective nucleic acid, and can, in fact, be "degenerate." Non-complementary bases or other sequences may be interspersed into the synthetic nucleic acid sequence, provided that the synthetic nucleic acid sequence has sufficient complementarity with the sequence to permit hybridization. Thus, by way of example, the primers used for PCR amplification may be selected to be "substantially" complementary to the specific sequence to be amplified.

As used herein, the term "hybridization" refers to the process by which a strand of nucleic acid joins with a complementary strand through base pairing as well as the process of amplification as carried out in, for example, PCR technologies. Nucleotide sequences that are capable of hybridizing to the complement of a given nucleotide sequence are generally functionally equivalent and can be substituted for that nucleotide sequence for the purposes of the methods described herein.

Thus, while this disclosure identifies specific primers and probes that have been found to be particularly sensitive and specific, persons of skill in the art would understand that useful primers include any primers that can prime a polymerase reaction at about the same locations as the exemplary primers disclosed herein. That is, primers which prime a polymerization reaction in exon 10 and exon 12 of the INSR gene can be used to amplify a diagnostic sequence. Primers

which span a the exon 10-exon 11 junction in the sequence of IR-B can be used to specifically amplify IR-B sequences. Similarly, additional probes which distinguish between IR-A and IR-B may be synthesized that specifically bind to amplified IR-A or IR-B target sequence. Generally, longer sequences comprising more complementary residues may contain greater variation.

"Stringent hybridization conditions" may be any of low stringency conditions, moderately stringent conditions and highly stringent conditions. Generally, "low stringency conditions" are, for example: hybridization in a solution comprising 5×SSC; 5× Denhart solution; 0.5% (w/v) SDS; and 50% (w/v) formamide; at 32° C. "Moderately stringent conditions" are, for example: hybridization in a solution comprising 5×SSC; SxDenhart solution; 0.5% (w/v) SDS; and 50% (w/v) formamide; at 42° C. "Highly stringent conditions" are, for example: hybridization in a solution comprising 5×SSC; SxDenhart solution; 0.5% (w/v) SDS; and 50% (w/v) formamide; at 50° C. Hybridization stringency is affected by a plurality of factors such as temperature, nucleic acid concentration, nucleic acid length, ion strength, time, and salt concentration. These are merely exemplary conditions that will produce the different levels of stringency. Those skilled in the art would be able to realize similar stringency by suitably adjusting hybridization conditions, including by adjusting such conditions for the desired stringency in a PCR reaction.

Synthetic nucleic acid sequences may be derived by cleavage of a larger nucleic acid fragment using non-specific nucleic acid cleaving chemicals or enzymes or site-specific restriction endonucleases; or by synthesis by standard methods known in the art, e.g. by use of a commercially available automated DNA synthesizer and standard phosphoramidite chemistry. One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Pat. No. 4,458,066.

Once a desired synthetic nucleic acid is synthesized, it can be cleaved from a solid support on which it was synthesized and treated, by methods known in the art, to remove any protecting groups present. The synthetic nucleic acids may then be purified by any method known in the art, including extraction and gel purification. The concentration and purity of the oligonucleotide may be determined by, for example, examining the oligonucleotide on an acrylamide gel, by HPLC, or by measuring the optical density at 260 nm in a spectrophotometer.

The synthetic nucleic acid sequences of the invention can be used in any assay which is used to determine for the presence of the expression of IR-A and IR-B. In one example, isolated nucleic acids such as disclosed herein can be used in an amplification process. Amplification refers to a process for multiplying nucleic acid strands in vitro. An exemplary technique is PCR, which exponentially amplifies nucleic acid molecules. PCR is described in U.S. Pat. No. 4,683,195 and U.S. Pat. No. 4,683,202. PCR is extensively used for specific detection and quantification of target nucleic acid sequences polynucleotides and is a standard method in molecular biology. PCR can be used to determine expression of IR-A and/or IR-B in a test sample. The method uses a pair of isolated nucleic acid sequences, "primers", which specifically anneal to specific locations on the IR-A or IR-B DNA molecule. The IR-A or IR-B DNA is heat denatured and two oligonucleotides that bracket the target sequence on opposite strands of the DNA to be amplified, are hybridized. These oligonucleotides become primers for use with DNA polymerase. The DNA is copied by primer extension to make a second copy of both strands. By repeating the cycle of heat denaturation,

primer hybridization and extension, the target IR-A or IR-B DNA can be amplified a million fold or more in about two to four hours. PCR is a molecular biology tool which must be used in conjunction with a detection technique to determine the results of amplification. An advantage of PCR is that it increases sensitivity by amplifying the amount of target DNA by 1 million to 1 billion fold in approximately 4 hours.

As discussed below and illustrated in the examples a useful method of using IR-A primers and probes is quantitative PCR. Quantitative PCR refers to methods where the PCR reaction is combined with fluorescence chemistry to enable real-time monitoring of the amplification reaction using detection of a fluorescent light signal. In one example the method uses a sequence nonspecific fluorescent reporter dye such as SYBR green (Wittwer C T et al., *Biotechniques*. 1997 January; 22(1):176-81). In another example, the method uses a sequence specific fluorescent reporter such as a TAQMAN probe (Heid C A et al., *Genome Res*. 1996 October; 6(10): 986-94). During execution of the PCR cycling program, the samples are excited using a light source. A fluorescent signal, indicating the amount of PCR amplification product produced, is monitored in each reaction well using a photodetector or CCD/CMOS camera. By monitoring the fluorescence in the sample during the reaction precise quantitative measurements can be made. The probe based PCR method is considered to more accurate than the SYBR green method. PCR or qPCR is typically performed in plastic 96 or 384 well microtiter plates, each reaction having a volume in the order of 5-50 µl. PCR can however be carried out in very small (nanoliter) volumes.

The term "primer" or "primer pair" as used herein refers to short oligonucleotides (typically 10-30 bp) which are used in PCR to prime a polymerization reaction. Specific primers may be used to select an IR-A or IR-B DNA sequence to be amplified by priming a polymerization at a specific location in the target sequence.

The methods described herein provide a method for the reproducible and robust amplification of small amounts of DNA which contain IR-A and/or IR-B. Performing qPCR using the nucleic acid primers described herein can specifically detect IR-A or IR-B from 0.1 picograms of DNA (1000 copies) or from 35 copies of the DNA.

A biological sample may comprise RNA that in some implementations of the method is first transcribed into cDNA. Total cellular RNA, cytoplasmic RNA, or poly(A)+RNA may be used. Methods for preparing total and poly(A)+RNA are well known and are described generally in Sambrook et al. (1989, *Molecular Cloning—A Laboratory Manual* (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) and Ausubel et al., eds. (1994, *Current Protocols in Molecular Biology*, vol. 2, Current Protocols Publishing, New York). Preferably, total RNA is prepared by the techniques described by Chirgwin et al. (1987), Chomczynski & Sacchi (1987), Sambrook et al. (1989), or Farrell Jr. (1993), and a number of high quality commercial kits are also available. More preferably, total RNA used is prepared using the guanidinium thiocyanate method of Chirgwin et al. (1987). The integrity of total RNA may be checked using various methods that are known in the art. By way of example, the RNA may be analyzed using RNA gel electrophoresis (e.g. formaldehyde/agarose gel), or Agilent LabChip. For mammalian total RNA, two bands at approximately 4.5 and 1.9 kb should be visible; these bands represent 28S and 18S ribosomal RNA respectively, and the ratio of intensities of these bands should typically be 1.5-2.5:1.

RNA purification kits for microscale RNA preparation are available from a number of commercial suppliers (for

example Absolutely RNA™ Nanoprep, Stratagene; PicoPure™, Arcturus; RNeasy®, Qiagen; RNAqueous™ Microkit, Ambion).

The cDNA synthesis oligonucleotide for first strand cDNA synthesis may be hybridized to RNA in a suitable buffer at a temperature between about 60° C. and 90° C., preferably about 70° C. for about 5 minutes, followed by cooling to about 4° C., before the reverse transcriptase is added. Following the hybridization of the cDNA synthesis oligonucleotide to RNA, a first cDNA strand is synthesized. This first strand of cDNA is preferably produced through the process of reverse transcription, wherein DNA is made from RNA, utilizing reverse transcriptase following methods that are familiar to a person skilled in the art.

Any reverse transcriptase may be used to transcribe RNA to DNA as long as the enzyme adds deoxyribonucleotides to the 3' terminus following extension (Varmus, Science 240: 1427-1435 (1988)) and the enzyme lacks RNaseH activity. Preferably, the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity such that longer cDNAs can be synthesized. The reverse transcriptase may be Moloney Murine Leukemia virus (MMLV) reverse transcriptase or a mutant thereof. The reverse transcriptase may be PowerScript™ Reverse Transcriptase (BD Biosciences Clontech). The reverse transcriptase may be SuperScript III™.

The amount of reverse transcriptase employed may vary as will be appreciated by a person skilled in the art. The reverse transcription is performed by incubation for, for example, approximately 1 hour with reverse transcriptase at an appropriate temperature, which must be in a temperature range in which the reverse transcriptase retains enzyme activity. The reaction may be performed between 37° C. and 55° C., preferably between 37° C. and 42° C. Most preferably, the reaction is performed at optimal enzyme activity—such as at about 42° C. The reverse transcription reaction may be terminated by heating the reaction mixture to 95° C. for about 5 minutes to inactivate the enzyme, optionally, followed by chilling on ice.

C. IGF Antagonists/Agonists

IGF agents as used herein refers to an agent that affects expression or activity of any member of the IGF-1R/IR signaling pathway and includes IGF-1R, IR, INSR, or IGF/II antagonists or agonists. The IGF-1R, IR, INSR, or IGF/II antagonist or agonist can be a peptidomimetic, protein, peptide, nucleic acid, small molecule, an antibody or other drug candidate. Examples of IGF-1R antagonists are well known in the art and include anti-sense and nucleic acids that antagonize IGF-1R have been described, e.g., in Wraight et al., Nat. Biotech., 18: 521-526 (2000); U.S. Pat. No. 5,643,788; U.S.

Pat. No. 6,340,674; US 2003/0031658; U.S. Pat. No. 6,340,674; U.S. Pat. No. 5,456,612; U.S. Pat. No. 5,643,788; U.S. Pat. No. 6,071,891; WO 2002/101002; WO 1999/23259; WO 2003/100059; US 2004/127446; US 2004/142895; US 2004/110296; US 2004/006035; US 2003/206887; US 2003/190635; US 2003/170891; US 2003/096769; U.S. Pat. No. 5,929,040; U.S. Pat. No. 6,284,741; US 2006/0234239; and U.S. Pat. No. 5,872,241.

Further, US 2005/0255493 discloses reducing IGF-1R expression by RNA interference using short double-stranded RNA. In addition, inhibitory peptides targeting IGF-1R have been generated that possess antiproliferative activity in vitro and in vivo (Pietrzkowski et al., Cancer Res., 52:6447-6451 (1992); Haylor et al., J. Am. Soc. Nephrol., 11:2027-2035 (2000)). A C-terminal peptide of IGF-1R has been shown to induce apoptosis and significantly inhibit tumor growth (Reiss et al., J. Cell. Phys., 181:124-135 (1999)). Also, a soluble form of IGF-1R inhibits tumor growth in vivo (D'Ambrosio et al., Cancer Res., 56: 4013-4020 (1996)). Small-molecule inhibitors to IGF-1R are described, e.g., in Garcia-Echeverria et al., Cancer Cell, 5: 231-239 (2004); Mitsiades et al., Cancer Cell, 5: 221-230 (2004); and Carboni et al., Cancer Res, 65: 3781-3787 (2005).

Further examples of disclosures on such small-molecule inhibitors include WO 2002/102804; WO 2002/102805; WO 2004/55022; U.S. Pat. No. 6,037,332; WO 2003/48133; US 2004/053931; US 2003/125370; U.S. Pat. No. 6,599,902; U.S. Pat. No. 6,117,880; WO 2003/35619; WO 2003/35614; WO 2003/35616; WO 2003/35615; WO 1998/48831; U.S. Pat. No. 6,337,338; US 2003/0064482; U.S. Pat. No. 6,475,486; U.S. Pat. No. 6,610,299; U.S. Pat. No. 5,561,119; WO 2006/080450; WO 2006/094600; and WO 2004/093781 See also WO 2007/099171 (bicyclo-pyrazole inhibitors) and WO 2007/099166 (pyrazolo-pyridine derivative inhibitors). See also (Hubbard et al., AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (October 22-26, San Francisco) 2007, Abst A227) on Abbott Corporation's molecule A-928605.

Examples of IGF agents include IGF-I/II agonists or antagonists. Specific IGF-II antagonists are also known in the art and include antibodies that bind IGF-I and/or IGF-II. Such antagonists are disclosed in WO 2007022172 and EP 492552. Examples of antibodies that bind both IGF-I and IGF-II include those described in WO2007070432, WO05/18671, WO 03/093317, WO 05/027970, and WO 05/028515.

A specific example of antibodies that are useful as IGF antagonists include those heavy and light chain components listed in Tables 1 and 2. These antibodies are disclosed in WO2007070432, which is incorporated by reference herein in its entirety. Particular antibodies include those designated 7.159.1, 7.158.1 and 7.34.1. The agents and antibodies disclosed above are incorporated into the present application in their entirety.

TABLE 1

Anti-IGF I/II Antibody Heavy Chain Analysis											
Chain Name	V	D	J	FR1	CDR 1			FR3	CDR3		
					FR2	CDR2			FR4		
Germline VH1-8		N.A.	JH6B	QVQLVQSGSYD	WVRQ	WMNPNS	RVTMTRNT	##YY	WGQG		
**				AEVKKPGA	IN	ATGQ	GNTGYA	SISTAYME	YYYG	TTVT	
SEQ ID				SVKVSCKA		GLEW	QKFQG	LSSLRSMDV	VSSA		
NO: 44				SGYTFT		MG		TAVYYCAR			
7.159.1 VH1-8		N.A.	JH6B	QVQLVQSGSYD	WVRQ	WMNPNS	RVTMTRNT	DPYY	WGQG		
SEQ ID				AEVKKPGA	IN	ATGQ	GNTGYA	SISTAYME	YYYG	TTVT	
NO: 45				SVKVSCKA		GLEW	QKFQG	LSSLRSMDV	VSSA		
				SGYTFT		MG		TAVYYCAR			

TABLE 1-continued

Anti-IGF I/II Antibody Heavy Chain Analysis									
Chain Name	V	D	J	FR1	CDR 1	FR2	CDR2	FR3	CDR3 FR4
Germline VH4-39 D6-19JH2				QLQLQESGSSSWIRQ	SIYYSG	RVTISVDT####	WGRG		
SEQ ID				PGLVKPSEYYWPPGK	STYYNP	SKNQFSLKSS##	TLVT		
NO: 46				TLSLTCTVG	GLEW	SLKS	LSSVTAADWYFD	VSSA	
				SGGSIS	IG		TAVYYCARL		
7.158.1 VH4-39 D6-19JH2				QLQLQESGSSSWIRQ	GIYYSG	RVTMSVDTQRGH	WGRG		
SEQ ID				PGLVKPSEYYWPPGK	STYYNP	SKNQFSLKSSGW	TLVT		
NO: 47				TLSLTCTVG	GLEW	SLKS	LSSVTAADWYFD	VSSA	
				SGGSIR	IG		TAVYYCARL		
7.234.1 VH4-39 D6-19JH2				QVQLQESGSSSWIRQ	GIYYSG	RVTMSVDTQRGH	WGRG		
SEQ ID				PGLVKPSENYWPPGK	STYYNP	SKNQFSLKSSGW	TLVT		
NO: 48				TLSLTCTVG	GLAW	SLRS	LSSVTAADWYFD	VSSA	
				SGGSIN	IG		TAVYYCARL		
Germline VH4-59 D1-20JH6B				QVQLQESGSYYWIRQ	YIYYSG	RVTISVDTITGT	WGQG		
SEQ ID				PGLVKPSEWS	PPGK	STNYNP	SKNQFSLK###G	TTVT	
NO: 49				TLSLTCTV	GLEW	SLKS	LSSVTAADMDV	VSSA	
				SGGSIS	IG		TAVYYCA#		
							R		
7.34.1 VH4-59 D1-20JH6B				QVQLQESGSYYWIRQ	YFFYSG	RVTMSVDTITGT	WGQG		
SEQ ID				PGLVKPSEWS	PPGR	YTNYNP	SKNQFSLKTKGG	ATVT	
NO: 50				TLSLTCTV	GLEW	SLKS	LSSVTAADMDV	VSSA	
				SGGSIS	IG		TAVYYCAC		
7.251.3 VH4-59 D1-20JH6B				QVQLQESGSYYWIRQ	YFFYSG	RVTISVDTITGT	WGQG		
SEQ ID				PGLVKPSEWS	PPGK	YTNYNP	SKNQFSLKTKGG	TTVT	
NO: 51				TLSLTCTV	GLEW	SLKS	LSSVTAADMDV	VSSA	
				SGGSIS	IG		TAVYYCAC		

* The hatch designation (#) indicates a space in the germline and is used to show a proper alignment with the antibody sequences shown in the table.

** The germline sequences shown in the above table are for alignment purposes, and it should be realized that each individual antibody region exists in its own location within the variable regions of immunoglobulin germline DNA segments in vivo.

TABLE 2

Anti-IGF I/II Antibody Light Chain Analysis									
Chain Name	V	J	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
Germline V1-19JL2			QSVLTQP	SGSSSN	WYQQLP	APKLLIY	GIPDRFSGSKS	GTWD	FGGG
SEQ ID			PSVSAAP	IGNNYV	GT	DNNKRPS	GTSATLGITGL	SSLS	TKLT
NO: 52			GQKVTIS	S			QTGDEADYYC	A##V	VLG
			C						
7.159.1 V1-19JL2			QSVLTQP	SGSSSN	WYQQLP	DNNKRPS	GIPDRFSGSKS	ETWD	FGGG
SEQ ID			PSVSAAP	IENNHV	GTAPKL		GTSATLGITGL	TSLS	TKLT
NO: 53			GQKVTIS	S	LIY		QTGDEADYYC	AGRV	VLG
			C						
Germline L5		JK3	DIQMTQS	RASQGI	WYQQKP	AASSLQS	GVPSRFSGSGS	QQAN	FGPG
SEQ ID			PSSVSAS	SSWLA	GKAPKL		GTDFTLTISSL	SFPF	TKVD
NO: 54			VGDRVTTI		LIY		QPEDFATYYC	T	IKR
			TC						
7.158.1 L5		JK3	DIQMTQS	RASQGI	WYQQKP	AASSLQS	GVPSRFSGSGS	QQAN	FGPG
SEQ ID			PSSVSAS	SSYLA	GKAPKL		GTDFTLTISSL	NFPF	TKVD
NO: 55			VGDSVTI		LIY		QPEDFATYYC	T	IKR
			TC						
7.234.1 L5		JK3	DIQMTQS	RASRGI	WYQORP	TASSLQS	GVPSRFSGSGS	QQAN	FGPG
SEQ ID			PSSVSAS	SSWLA	GKAPKL		GTDFTLTISSL	SFPF	TKVD
NO: 56			VGDRVTTI		LIY		QPEDFATYYC	T	IKR
			TC						
Germline V1-13JL2			QSVLTQP	TGSSSN	WYQQLP	GNSNRPS	GVPDRFSGSKS	QSYD	FGGG
SEQ ID			PSVSGAP	IGAGYD	GTAPKL		GTSASLAITGL	SSLS	TKLT
NO: 57			GQRVTTIS	VH	LIY		QAEDADYYC	GSV	VLG
			C						

TABLE 2-continued

Anti-IGF I/II Antibody Light Chain Analysis									
Chain Name	V	J	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
7.34.1	V1-13JL2	QSVLTQA	TGRSSN	WYQQFP	GNSNRPS	GVPDRFSGSKS	QSYD	FGGG	
SEQ ID		PSVSGAP	IGAGYD	GTAPKL		GTSASLAITGL	SSLS	TKLT	
NO: 58		GQRVTIS	VH	LIY		QAEDEADYYC	GSV	VLG	
		C							
7.251.3	V1-13JL2	QSVLTQP	TGSSSN	WYQQLP	GNNNRPS	GVPDRFSGSKS	QSPD	FGGG	
SEQ ID		PSVSGAP	IGAGYD	GTAPKL		GTSASLAITGL	SSLS	TKLT	
NO: 59		GQRVTIS	VH	LIY		QADDEADYYC	GSV	VLG	
		C							

* The hatch designation (#) indicates a space in the germline and is used to show a proper alignment with the antibody sequences shown in the table.

** The germline sequences shown in the above table are for alignment purposes, and it should be realized that each individual antibody region exists in its own location within the variable regions of immunoglobulin germline DNA segments in vivo.

D. Methods of Detecting and/or Quantifying IR-A and IR-B Expression

The IR-A and IR-B synthetic nucleic acid sequences, primers and probe sets disclosed above can be used to determine the level of IR-A or IR-B in a sample. Given the sensitivity of the assay, the molecules of the present invention can have numerous uses.

A preferred approach is to use a real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction (variously abbreviated Q-PCR, qPCR, qrt-PCR, or RTQ-PCR) or kinetic polymerase chain reaction (KPCR). Frequently, real-time PCR is combined with reverse transcription to quantify messenger RNA and non-coding RNA in cells or tissues. Reverse transcription PCR permits starting from an RNA containing sample without prior preparation of cDNA. Real-time reverse-transcription PCR is often denoted as qRT-PCR, RRT-PCR, or RT-rt PCR. It enables both detection and quantification (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes) of one or more specific sequences in a DNA sample.

The procedure follows the general course of a polymerase chain reaction. However, amplified DNA is detected as the reaction progresses in real time. Two common methods for detection of products in real-time PCR are: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary DNA target.

Fluorescent reporter probes detect only the DNA containing the probe sequence; therefore, use of the reporter probe significantly increases specificity, and enables quantification even in the presence of non-specific DNA amplification. Fluorescent probes can be used in multiplex assays—for detection of several genes in the same reaction—based on specific probes with different-colored labels, provided that all targeted genes are amplified with similar efficiency.

The method generally uses a DNA-based probe with a fluorescent reporter at one end and a quencher of fluorescence at the opposite end of the probe. The close proximity of the reporter to the quencher prevents detection of its fluorescence; breakdown of the probe by the 5' to 3' exonuclease activity of a polymerase separates the reporter from the quencher and thus allows unquenched emission of fluorescence. An increase in the product targeted by the reporter

probe at each PCR cycle therefore causes a proportional increase in fluorescence due to the breakdown of the probe and release of the reporter.

A PCR sample is prepared as usual, and the reporter probe is added. As the reaction commences, during the annealing stage of the PCR both probe and primers anneal to the DNA target.

Polymerization of a new DNA strand is initiated from the primers, and once the polymerase reaches the probe, its 5'-3'-exonuclease degrades the probe, physically separating the fluorescent reporter from the quencher, resulting in an increase in fluorescence. Fluorescence is detected and measured in the real-time PCR thermocycler, and its geometric increase corresponding to exponential increase of the product is used to determine the threshold cycle (CT) in each reaction.

Relative concentrations of DNA present during the exponential phase of the reaction can be determined by plotting fluorescence against cycle number on a logarithmic scale (so an exponentially increasing quantity will give a straight line). A threshold for detection of fluorescence above background is determined. The cycle at which the fluorescence from a sample crosses the threshold is called the cycle threshold, Ct. The quantity of DNA theoretically doubles every cycle during the exponential phase and relative amounts of DNA can be calculated, e.g. a sample whose Ct is 3 cycles earlier than another's has $2^3=8$ times more template. Since all sets of primers don't work equally well, one has to calculate the reaction efficiency first. Thus, by using this as the base and the cycle difference C(t) as the exponent, the difference in starting template can be calculated as $(2 \times \% \text{ eff})^{Ct}$.

Amounts of RNA or DNA can then be determined by comparing the results to a standard curve produced by real-time PCR of serial dilutions (e.g. undiluted, 1:4, 1:16, 1:64) of a known amount of RNA or DNA. To accurately quantify gene expression, the measured amount of RNA from the gene of interest is divided by the amount of RNA from a house-keeping gene measured in the same sample to normalize for possible variation in the amount and quality of RNA between different samples. This normalization permits accurate comparison of expression of the gene of interest between different samples, provided that the expression of the reference gene used in the normalization is very similar across all the samples.

Mechanism based qPCR quantification methods have also been described, such as MAK2. They do not require a standard curve for quantification. These mechanism based meth-

ods use knowledge about the polymerase amplification process to generate estimates of the original sample concentration.

Real-time PCR can be used to determine relative quantities and absolute quantities. Relative quantification measures the fold-difference ($2\times$, $3\times$ etc.) in the target amount. Absolute quantification gives the exact number of target molecules present by comparing with known standards.

E. Diagnostic Classification of a Tumor

A method of classifying a tumor can comprise providing a tumor sample; contacting the sample with a synthetic IR-A specific oligonucleotide; and detecting or quantifying the amount of IR-A in the tumor. A quantification of IR-A in the tumor may be compared to a control tissue sample or to a population average for normal tissue. For example, a breast cancer tumor sample may be compared to a sample from non-affected breast of the same patient or to a population average for non-affected breast tissue. Increased expression of IR-A indicates that the tumor is an IR-A expressing tumor.

A method of classifying a tumor can alternatively include detecting and/or quantifying the amount of IR-B in a tumor compared to a control sample or a population average. Thus, a method of classifying a tumor can comprise providing a tumor sample; contacting the sample with a synthetic IR-B specific oligonucleotide; and detecting or quantifying the amount of IR-B in the tumor. A quantification of IR-B in the tumor may be compared to a control tissue sample or to a population average for normal tissue. For example, a breast cancer tumor sample may be compared to a sample from a non-affected breast of the same patient or to a population average for non-affected breast tissue. Increased expression of IR-B indicates that the tumor is an IR-B expressing tumor.

A method of classifying a tumor can include determining the relative expression levels of IR-A and IR-B. The relative expression can be described as a ratio of IR-A:IR-B mRNA, or as a percentage of either as a proportion of the total IR mRNA, e.g., % IR-A. The relative expression of IR-A and IR-B can also be described by the differential of the threshold cycles in a qPCR, e.g. $(IR-A \Delta Ct) - (IR-B \Delta Ct) = \Delta \Delta Ct$, the ratio of IR-A mRNA to IR-B mRNA in the sample being approximately equal to $2^{-\Delta \Delta Ct}$. For ΔCt calculation, the experimental Ct values can be normalized against an internal standard. For example, a mean of in-sample (i.e., obtained from the same sample as IR-A and/or IR-B expression) Ct values of a gene expression panel, such as the average Ct of one or more housekeeping genes, can be used for normalization of Ct values for IR-A and IR-B to calculate ΔCt values.

To classify a tissue sample, such as a tumor tissue sample, based upon the percentage of IR-A relative to total INSR expression, the mean and standard deviation of the percentage of IR-A in the tissue type is first determined. In another approach, the confidence range of the mean is statistically determined for a pre-selected confidence interval, e.g. 95%, 97.5%, 99% or 99.9% confidence. The percentage of IR-A relative to total INSR expression in the tissue sample is then determined in the tissue sample. If the percentage of IR-A out of the total INSR expression is greater than the mean, then the tissue may be said to have an elevated proportion of IR-A expression. If the proportion of IR-A out of the total INSR expression is greater than 1 to 3 standard deviations, e.g. greater than 2 standard deviations then the sample may be said to have a substantially elevated proportion of IR-A. Similarly, if the proportion of IR-A out of the total INSR expression is greater than the upper confidence interval of the mean value for a pre-selected confidence level, then the tissue may

be said to have an abnormally high proportion of IR-A. A classification may be assigned to a tissue sample that exceeds 1, 2, or 3 times the 95%, 97.5%, 99% or 99.9% confidence interval of a mean value for a type of tissue.

Alternatively, a mean percentage of IR-A mRNA relative to total INSR mRNA and a confidence interval for a pre-selected confidence level may be determined for a known classification of tumor. If a measured IR-A percentage of a tissue sample falls within the confidence interval, the measurement may be said to be consistent with the tumor classification.

As an example, normal breast tissue has been determined to contain $46.6 \pm 4.7\%$ IR-A mRNA as a proportion of total INSR mRNA at 95% confidence. Breast tumor tissue has been found to contain 75.24% IR-A relative to total INSR mRNA with a 95% confidence interval of $\pm 5\%$. These measurements have been determined to be significantly different ($p < 0.0001$). A tissue sample IR-A percentage greater than 46.6% could be said to indicate a higher than average percentage of IR-A. However, setting a threshold of about 60%, nearly mid-point between the mean values of normal and tumor tissue and well beyond the respective 95% confidence ranges will minimize the number of incorrectly classified samples. A skilled practitioner may adjust the threshold in the range 47-75%, for example selecting a threshold in the range 55% to 65%, to favor a more or less inclusive classification.

As another example IR-A and IR-B ΔCt values may be determined for normal tissue of any given type. Tissue samples determined to have $(IR-A \Delta Ct) - (IR-B \Delta Ct)$ differentials that are more than 1, 2, 3 or more standard deviations below the mean value may be classified as having disproportionate levels of IR-A expression relative to IR-B expression. By contrast, a positive ΔCt differential indicates a disproportionate level of IR-B expression. To illustrate, IR-A:IR-B ΔCt differentials were determined in normal and primary tumor breast samples. The mean IR-A:IR-B $\Delta \Delta Ct \pm 95\%$ CI was 0.20 ± 0.23 for normal ($n=19$) and the mean IR-A:IR-B $\Delta \Delta Ct \pm 95\%$ CI was -1.81 ± 0.27 in primary tumors ($n=42$). Thus, a tissue sample having a IR-A:IR-B $\Delta \Delta Ct$ about 0.2 may be said to have a higher than average IR-A:IR-B $\Delta \Delta Ct$, indicating a higher than average proportion of IR-A expression. However, setting a threshold of about -0.4, -0.6, -0.8, -1.0 or -1.2 would provide increasing levels of confidence. Setting a threshold near the midpoint (in this example at an IR-A:IR-B $\Delta \Delta Ct$ of about -0.7 to -0.9) would minimize the number of incorrect classifications. Of course, a skilled practitioner may adjust the threshold anywhere in the range between mean values to balance the needs of the classification to be more or less inclusive. Thus, a threshold for classifying a sample as having IR-A:IR-B $\Delta \Delta Ct$ differentials consistent with breast tumor tissue having an altered amount of IR-A relative to IR-B may be set in the range 0.2 to -1.8, for example between about 0.4 and about -1.54, based on the 95% confidence interval.

As another example, relative expression of IR-A and IR-B may be used to classify tumor subtypes, for example, luminal A and luminal B breast cancers. For example, IR-A:IR-B ΔCt differentials in the normal, luminal A and luminal B were compared. The mean IR-A:IR-B $\Delta \Delta Ct \pm 95\%$ CI was 0.27 ± 0.30 in normal ($n=15$). The mean IR-A:IR-B $\Delta \Delta Ct \pm 95\%$ CI was -1.09 ± 0.34 in luminal A classified breast cancers ($n=13$). The mean IR-A:IR-B $\Delta \Delta Ct \pm 95\%$ CI was -2.12 ± 0.34 in luminal B classified breast cancers ($n=27$). All subtype pair-wise comparisons display a significant difference (two-sample t-test, $p < 0.001$). Accordingly, a threshold for IR-A:IR-B $\Delta \Delta Ct$ classification between normal and luminal A tumor tissue may be set in the range between about 0.3

to -1.4, for example at about -0.2, about -0.4, or about -0.6. A threshold for IR-A:IR-B $\Delta\Delta\text{Ct}$ classification between luminal A and luminal B tumor tissue may be set in the range between about -1.1 to about -2.1, for example in the range between about -1.5 to -1.75, or at about -1.55, about -1.6, about -1.65, or about -1.7.

Using a classification scheme for normal, luminal-A, and luminal-B, based upon GeneChip expression profiles, IR-A:IR-B ΔCt differentials in normal, luminal-A and luminal-B classified tumor samples were compared. The mean IR-A:IR-B $\Delta\Delta\text{Ct}\pm 95\%$ CI was 0.32 ± 0.25 in normal ($n=15$). The mean IR-A:IR-B $\Delta\Delta\text{Ct}\pm 95\%$ CI was -1.05 ± 0.19 in luminal-A predicted breast cancers ($n=18$). The mean IR-A:IR-B $\Delta\Delta\text{Ct}\pm 95\%$ CI was -2.42 ± 0.32 in luminal-B predicted breast cancers ($n=22$). In accordance with this scheme, a threshold for IR-A:IR-B $\Delta\Delta\text{Ct}$ classification between luminal A and luminal B tumor tissue may be set in the range between about -1.1 to about -2.4, for example in the range between about -1.4 to -2.1, or at about -1.4, about -1.6, about -1.7, about -1.8, or about -1.9. IR-A:IR-B $\Delta\Delta\text{Ct}$ may be combined with other expression profiles to further refine subtype classifications.

Relative IR-A and IR-B expression levels in tissues may be used as a predictor of cancer proliferation, particularly in combination with other predictors of cancer proliferation, for example to determine a proliferation score. Predicted proliferation rates can provide useful information on prognosis and aggressiveness of individual cancers. The data above illustrate a positive correlation between the IR-A:IR-B ΔCt differential and the proliferation score. Thus, a method for scoring tumor tissue may comprise determining the relative proportion of IR-A and IR-B expression and assigning a proliferation score based at least in part upon the relative expression of IR-A and IR-B.

Tumor samples for classification using these methods can be any appropriate tumor sample including a sample from a lung, breast, prostate, colon, ovary, pancreas, brain, esophagus, endometrium, cervix, gastrointestinal tract or skin. Tumor samples can be taken from any patient where the tumor activity is mediated alone, or in part, through a cell surface receptor such as IGF-R1/IR-A. For example, the tumor can be a non-solid tumor such as leukemia, multiple myeloma or lymphoma, or can be a solid tumor, for example bile duct, bone, bladder, brain/CNS, breast, colorectal, cervical, endometrial, gastric, head and neck, hepatic, lung, muscle, neuronal, esophageal, ovarian, pancreatic, pleural/peritoneal membranes, prostate, renal, skin, testicular, thyroid, uterine and vulval tumors. In one example, the tumor is a tumor of the breast. In another example, the tumor is of the bladder. In another example, the tumor is of the liver.

Appropriate tumor samples can be prepared as known in the art. For example, live tumor cells are obtained via a needle biopsy and then cultured in vitro according to standard procedures. Alternately, one could fix the tumor cells immediately following aspiration or remove the tumor (in whole or in part) and prepare a section for immunohistological staining.

In vitro culturing of tumor cells will enable the measurement of internalization dynamics following stimulation, while immediately fixing samples will result in assaying the static localization of the receptor within the tumor.

F. Methods of Treatment

The IGF-1 receptor (IGF-IR) pathway is complex and includes multiple players (see FIG. 3). IGF-1R can and does form hybrid receptors with the insulin receptor (INSR). IGF ligands, IGF-I and IGF-II, exert their various actions by pri-

marily interacting with IGF-IR and activating various intracellular signaling cascades. Specifically, IGF-I functions primarily by activating the IGF-IR, whereas IGF-II can act through either the IGF-IR or through the IR-A isoform. IGF-1R, its IGF ligands and IR-A are implicated in numerous cancers including both breast and prostate cancer and numerous antagonists for use in cancer treatment have been developed to target IGF-IR and the IGF ligands.

Given the multitude of antagonists currently available that target the IGF pathway, the selection of an antagonist to which a patient is likely to respond or has heightened response is desired. For example, IGF-1R antagonists suffer from the limitation that these antagonists do not inhibit the IR-A pathway. Given that the literature suggests that IR-A when overexpressed in cancer can be responsible for resistance to IGF-1R antagonists, it is desirable to administer an antagonist that targets not just IGF-1R but also IR-A. An example of such an antagonist in an antibody that specifically targets IGF-II and can cross-react with IGF-I.

These IGF-I and II antagonists have the ability to inhibit both IGF-IR and IR-A signaling, resulting in a broader activity in the clinic than IGF-1R and reduced toxicity compared to small molecule IGF-1R/IR-A/IR-B inhibitors. Examples include the antibodies against IGF-I and/or IGF-II, including those disclosed in WO2007070432. Of particular interest are antibodies having the amino acid sequence of the antibody produced by hybridomas 7.159.1, 7.158.1 and 7.34.1.

A method of selecting patients who are candidates for treatment with an IGF antagonist or agonist in order to predict an increased likelihood of response to a particular IGF antagonist or agonist can comprise quantifying IR-A and/or IR-B expression using any of the above methods and selecting a patient who expresses an increased or decreased amount of IR-A and/or IR-B relative to normal subjects or relative to a population of cancer patients. Patients may also be selected according to altered relative amounts of IR-A versus IR-B expression.

In a specific example, a practitioner may pre-select a particular IGF antagonist based on the determination if the tumor expresses IR-A and/or IR-B. The identification of a tumor that has been determined to overexpress IR-A provides the opportunity to select patients that will most likely have increased responsiveness to an IGF-I/II antagonist.

In one example, the antagonist is an antibody comprising an amino acid sequence comprising the amino acid sequences of SEQ ID NOs: 45 and 53. In another example, the antagonist is an antibody comprising the CDR sequences of SEQ ID NOs: 45 and 53, as shown in Tables 1 and 2. In another example, the antagonist comprises an antibody comprising three CDRs from SEQ ID NO: 45 and a light chain. In another example, the antagonist comprises an antibody comprising three CDRs from SEQ ID NO: 53 and a heavy chain.

The method can also be used to determine if a particular inhibitor of IGF-1R or IGF is activating or inhibiting the insulin receptor. For example, it is known that small molecule inhibitors of the IGF-1R kinase often cross-inhibit the insulin receptor. This can lead to metabolic complications. In one example, a method determining for the expression of IR-A and/or IR-B in a sample such as a tumor and comparing their expression to a control. If the expression of IR-B is decreased compared to a control and expression of IR-A is increased compared to a control, then alternative selection of an IGF antagonist may be required such as an IGF-I/II antagonist. Thus, a method of treating a patient may comprise determining the relative expression of IR-A and IR-B, for example by determining an IR-A:IR-B ΔCt differential for a tissue

sample from a patient, and administering an IGF-I/II antagonist if the proportion of IR-A relative to IR-B is lower than a threshold value.

In another example, a method allows for the classification of a subset of cancer patients. Currently it is known that IR-A may be overexpressed in breast cancer. A method of selecting a subset of patients for treatment may comprise identification of a subset of breast cancer patients who overexpress IR-A, or who express IR-A disproportionately relative to IR-B, and who are therefore likely to have a heightened response to an IGF-I/II antagonist. A method of treating cancer patients who are likely to have a heightened response to an IGF-I/II antagonist can comprise measuring an IR-A:IR-B Δ Ct differential for a tumor tissue sample and administering an effective dose of an IGF-I/II antagonist if the IR-A:IR-B Δ Ct differential of the tumor tissue sample is below a threshold value indicating a higher than normal proportion of IR-A relative to IR-B. Examples of antagonists include antibodies that bind IGF-I and/or IGF-II. In one example, the antagonist is an antibody comprising an amino acid sequence comprising the amino acid sequences of SEQ ID NOs: 45 and 53. In another example, the antagonist is an antibody comprising the CDR sequences of SEQ ID NOs: 45 and 53, as shown in Tables 1 and 2. In another example, the antagonist comprises an antibody comprising three CDRs from SEQ ID NO: 45 and a light chain. In another example, the antagonist comprises an antibody comprising three CDRs from SEQ ID NO: 53 and a heavy chain.

By heightened response or responders it is meant patients that will respond, or respond more positively, following administration of a particular IGF agent. Responders and non responders can be determined by measuring objective tumor responses according to the Union International Contre le Cancer/World Health Organization (U ICC/WHO) criteria. The criteria are categorized as follows: complete response (CR): no residual tumor in all evaluable lesions; partial response (PR): residual tumor with evidence of chemotherapy-induced 50% or greater decrease under baseline in the sum of all measurable lesions and no new lesions; stable disease (SD): residual tumor not qualified for CR; and progressive disease (PD): residual tumor with evidence of 25% or greater increase under baseline in the sum of all measurable lesions or appearance of new lesions. As defined herein non-responders are PD. The methods are particularly effective for determining those patients that are CR or PR. The methods thus permit improved prognosis and quality of life of cancer patients by matching the treatments to individual patients and so making more effective use of the types of IGF antagonists available.

G. Diabetes

A method for screening for substances/compounds that abolish and/or decrease signaling via insulin receptors. Determining the relative expression of IR-A or IR-B in a sample can be used as a screening tool to identify agents such as small molecule compounds and/or insulin mimetics, that selectively activate either IR-A or IR-B-specific signaling cascades, e.g. in the beta cell and in peripheral tissues. The pronounced expression of IR-B in the classical insulin target tissues indicates the importance of the IR-B signaling cascade in these tissues. Consequently compounds that selectively stimulate the IR-B signaling cascade will improve the function of the beta cell (glucose responsiveness and therefore insulin secretion), as well as the function of the peripheral insulin target tissues (glucose uptake and utilization, protein synthesis, lipid synthesis) and thus potentially provide a treat-

ment that covers the two major causes of non-insulin dependent diabetes mellitus (NIDDM, type II diabetes), i.e. peripheral insulin resistance and beta cell dysfunction.

Thus, methods of identifying an agent which modulates insulin signaling can include contacting a cell with a test agent and determining if that test agent results in an increase or decreases in IR-B expression. The identification of an agent that increases IR-B expression is indicative that the agent can be useful in treating type II diabetes.

H. Kits

Kits for detecting the presence of IR-A or IR-B in a biological sample may comprise an IR-A and/or IR-B probe or primer. Materials for use in the methods described herein are ideally suited for preparation of kits. For example, the kit can comprise nucleic acid sequences as disclosed herein that are capable of detecting IR-A or IR-B in a tumor sample; a control sample; and instructions relating to how to detect the cell surface receptor. Such a kit may comprise containers, each with one or more of the various reagents (typically in concentrated form) utilized in the methods, including, for example, buffers, the appropriate nucleotide triphosphates (e.g., dATP, dCTP, dGTP and dTTP; or rATP, rCTP, rGTP and UTP), reverse transcriptase, DNA polymerase, RNA polymerase, and one or more oligonucleotides.

Oligonucleotides in containers can be in any form, e.g., lyophilized, or in solution (e.g., a distilled water or buffered solution), etc. Oligonucleotides ready for use in the same amplification reaction can be combined in a single container or can be in separate containers. The kit optionally further comprises in a separate container an RNA polymerase specific to the RNA polymerase promoter, and/or a buffer for PCR, and/or a DNA polymerase. The kit optionally further comprises a control nucleic acid. A set of instructions will also typically be included.

The methods disclosed herein employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; and, D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA Methods in Enzymology*, Academic Press. Each of these general texts is herein incorporated by reference in their entireties.

The following examples are meant to serve to assist one of ordinary skill in the art in carrying out the methods described herein and are not intended in any way to limit the scope of the disclosure.

I. Exemplary Embodiments

The following list of embodiments is exemplary, and in no way limits the scope of the disclosure.

1. A synthetic nucleic acid comprising 10-30 consecutive nucleotides, wherein the synthetic nucleic acid sequence comprises at least 10-20 consecutive nucleotides of any one of the following sequences:

- (i) SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions;
 - (ii) SEQ ID NO: 4, a sequence complementary thereto (SEQ ID NO: 21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions;
 - (iii) SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions;
 - (iv) SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO: 22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions.
2. A synthetic nucleic acid sequence consisting essentially of any one of the following nucleic acid sequences:
- (i) SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions;
 - (ii) SEQ ID NO: 4, a sequence complementary thereto (SEQ ID NO: 21), or a sequence that is capable of hybridizing to SEQ ID NO:4, or its complement, under stringent conditions;
 - (iii) SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5 or its complement, under stringent conditions; or
 - (iv) SEQ ID NO: 6, a sequence complementary thereto (SEQ ID NO: 22), or a sequence that is capable of hybridizing to SEQ ID NO:6, or its complement, under stringent conditions.
3. A synthetic nucleic acid sequence consisting essentially of SEQ ID NO: 7, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions.
4. A synthetic nucleic acid sequence consisting essentially of SEQ ID NO: 8, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions.
5. A composition comprising the synthetic nucleic acid sequence of any one of embodiments 1-4.
6. A primer set for detecting and/or quantifying an IR-A nucleic acid sequence in a biological sample, wherein the primer set comprises
- (a) a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of the last 50 bases of exon 10 of the INSR gene (SEQ ID NO: 1), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:1, or its complement, under stringent conditions; and
 - (b) a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of the first 60 bases of exon 12 of the INSR gene (SEQ ID NO: 2), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:2, or its complement, under stringent conditions.
7. The primer set of embodiment 6, wherein the at least one synthetic nucleic acid sequence has a nucleotide sequence chosen from among
- SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions;
 - SEQ ID NO: 21, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:21, or its complement, under stringent conditions;
 - SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions; and

- SEQ ID NO: 22, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:22, or its complement, under stringent conditions.
8. A method for detecting and/or quantifying an IR-A nucleic acid sequence in a biological sample, comprising the steps of:
- (a) contacting a biological sample or nucleic acids prepared from a biological sample with the primer set of embodiment 6 under conditions suitable for polymerase-based amplification; and
 - (b) detecting and/or quantifying amplified target IR-A nucleic acid sequence.
9. The method of embodiment 8, wherein the biological sample is prepared from a tumor sample.
10. The method of any one of the embodiments 8-9, wherein the primer set comprises SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions, and SEQ ID NO: 21, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:21, or its complement, under stringent conditions.
11. The method of embodiment 8, wherein the primer set comprises SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions, and SEQ ID NO: 22, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:22, or its complement, under stringent conditions.
12. The method of any of embodiments 8-11, wherein said polymerase-based amplification is quantitative polymerase chain reaction.
13. The method of embodiment 12, wherein the primer set comprises:
- SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions;
 - SEQ ID NO: 21, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:21, or its complement, under stringent conditions; and
 - SEQ ID NO: 7, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions, which also has a detectable label.
14. The method of embodiment 12, wherein the primer set comprises:
- SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions;
 - SEQ ID NO: 22, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:22, or its complement, under stringent conditions; and
 - SEQ ID NO: 8, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions, which also has a detectable label.
15. The method of any of embodiments 8-14, wherein the amplified product is less than 100 bases.
16. A synthetic nucleic acid comprising 10-30 consecutive nucleotides, wherein the synthetic nucleic acid sequence comprises at least 10-20 consecutive nucleotides of any one of the following sequences:

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(i) SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; or

(ii) SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions.

17. A synthetic nucleic acid consisting essentially of any one of the following nucleic acid sequences:

(i) SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; or

(ii) SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions.

18. A synthetic nucleic acid sequence consisting essentially of SEQ ID NO: 13, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:13, or its complement, under stringent conditions.

19. A synthetic nucleic acid sequence consisting essentially of SEQ ID NO: 14, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:14, or its complement, under stringent conditions.

20. A synthetic nucleic acid sequence consisting essentially of SEQ ID NO: 15, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:15, or its complement, under stringent conditions.

21. A composition comprising the synthetic nucleic acid sequence of any one of embodiments 1-4 and 16-20.

22. A primer set for detecting and/or quantifying an IR-B nucleic acid sequence in a biological sample, wherein the primer set comprises:

(a)(i) a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of the last 50 bases of exon 10 of the INSR gene (SEQ ID NO: 1), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:1, or its complement, under stringent conditions,

(a)(ii) a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of exon 11 of the INSR gene (SEQ ID NO: 9), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:9, or its complement, under stringent conditions; or

(a)(iii) a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of the bases bridging exons 10 and 11, a sequence complementary thereto, or a sequence that is capable of hybridizing thereto, or its complement, under stringent conditions; and

(b) a synthetic nucleic acid sequence comprising 10-30 consecutive nucleotides of the first 50 bases of exon 12 of the INSR gene (SEQ ID NO: 10), a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:10, or its complement, under stringent conditions or a sequence complementary thereto.

23. The primer set of embodiment 22, wherein the at least one synthetic nucleic acid sequence has a nucleotide sequence chosen from among

SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; and

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions.

24. A method for detecting and/or quantifying a IR-B nucleic acid in a biological sample, comprising the steps of:

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(a) contacting a biological sample, or nucleic acids prepared from a biological sample, with the primer set of embodiment 22 or 23 under conditions suitable for polymerase-based amplification; and

(b) detecting and/or quantifying amplified target IR-B nucleic acid sequence.

25. The method of embodiment 24, wherein the biological sample is a tumor sample.

24. The method of embodiment 24, wherein the primer set comprises:

SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions; and

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions.

25. The method of embodiment 24, wherein said polymerase-based amplification is quantitative polymerase chain reaction (q-PCR).

26. The method of embodiment 25, wherein the primer set comprises:

SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions;

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and

SEQ ID NO: 13, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:13, or its complement, under stringent conditions which also has a detectable label.

27. The method of embodiment 25, wherein the primer set comprises:

SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions;

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and

SEQ ID NO: 14, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:14, or its complement, under stringent conditions which also has a detectable label.

28. The method of embodiment 25, wherein the primer set comprises:

SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions;

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and further comprises

SEQ ID NO: 15, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:15, or its complement, under stringent conditions, which also has a detectable label.

29. The method of any of embodiments 24-28, wherein the amplified product is less than 100 bases.

30. A method for determining the presence or absence of a target IR-A nucleic acid sequence and/or a target IR-B nucleic acid sequence in a biological sample, comprising:

contacting a biological sample, or a sample of nucleic acids prepared from a biological sample, with a primer set for detecting and/or quantifying an IR-A nucleic acid

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sequence in a biological sample under conditions suitable for polymerase-based amplification, wherein the primer set comprises the primer set of embodiment 6 and/or contacting a biological sample, or a sample of nucleic acids prepared from a biological sample, with a primer set for detecting and/or quantifying an IR-B nucleic acid sequence in a biological sample under conditions suitable for polymerase-based amplification, wherein the primer set comprises the primer set of embodiment the primer set of embodiment 22, and detecting and/or quantifying amplified target IR-A or IR-B nucleic acid sequence.

31. A kit for determining the presence or absence of IR-A in a biological sample comprising at least one synthetic nucleic acid sequence of any of embodiments 1-15 and instructions for use.

32. The kit of embodiment 31, wherein the at least one synthetic nucleic acid sequence has a nucleotide sequence chosen from among SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, a sequence complementary to one of said sequences, or a sequence that is capable of hybridizing to one of said sequences, or its complement, under stringent conditions.

33. The kit of embodiment 31, wherein the synthetic nucleic acid sequence is chosen from among the following primer sets:

- (1) SEQ ID NO: 3, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:3, or its complement, under stringent conditions; SEQ ID NO: 21, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:21, or its complement, under stringent conditions; and SEQ ID NO: 7, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:7, or its complement, under stringent conditions, which also has a detectable label; or
- (2) SEQ ID NO: 5, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:5, or its complement, under stringent conditions; SEQ ID NO: 22 a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:22, or its complement, under stringent conditions; and
- SEQ ID NO: 8, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:8, or its complement, under stringent conditions, which also has a detectable label.

34. The kit of embodiment 31, further comprising suitable PCR reagents; and optionally, a positive and/or negative control for determining the presence or absence of IR-A.

35. A kit for determining the presence or absence of IR-B in a biological sample comprising at least one synthetic nucleic acid sequence of any of embodiments 16-23 and instructions for use.

36. The kit of embodiment 35, wherein the synthetic nucleic acid sequence has a nucleotide sequence chosen from among SEQ ID NO:11, SEQ ID NO:12, a sequence complementary to any of said sequences, or a sequence that is capable of hybridizing to any of said sequences, or its complement, under stringent conditions.

37. The kit of embodiment 35, wherein the synthetic nucleic acid sequence is chosen from the following primer sets:

- (1) SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions;

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SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and

SEQ ID NO: 13, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:13, or its complement, under stringent conditions having a detectable label;

(2) SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions;

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and

SEQ ID NO: 14 having a detectable label; or

(3) SEQ ID NO: 11, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:11, or its complement, under stringent conditions;

SEQ ID NO: 12, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:12, or its complement, under stringent conditions; and

SEQ ID NO: 15, a sequence complementary thereto, or a sequence that is capable of hybridizing to SEQ ID NO:15, or its complement, under stringent conditions, which also has a detectable label.

38. The kit of embodiment 35, further comprising: suitable PCR reagents; and optionally, a positive and/or negative control for determining the presence or absence of IR-B.

39. A method for selecting a patient responsive to an IGF1/II ligand or IGF1R receptor antagonist, the method comprising:

detecting and/or quantifying IR-A expression in a sample according to embodiment 8

detecting and/or quantifying IR-B expression in a sample according to embodiment 24; and

wherein the expression of IR-A and IR-B is an indication of whether an IGF1/II ligand or IGF1R receptor antagonist should be administered to the subject.

40. The method of embodiment 39, wherein the IGF1/II ligand or IGF1R receptor antagonist is an antibody.

41. The method of embodiment 40, wherein the antibody comprises sequence components listed in Table 1 and Table 2.

42. A method for determining the relative presence or absence of a target IR-A nucleic acid sequence and a target IR-B nucleic acid sequence in a biological sample, comprising:

contacting a biological sample or nucleic acids prepared from a biological sample, under conditions suitable for polymerase-based amplification, with the primer set of embodiment 6,

contacting a biological sample or nucleic acids prepared from a biological sample, under conditions suitable for polymerase-based amplification, with a primer set of embodiment 22;

quantifying amplified IR-A and IR-B nucleic acid sequence; and

thereby determining a relative expression of IR-A versus IR-B in the biological sample.

43. A method of classifying a tumor comprising determining a relative expression of IR-A versus IR-B in a sample of the tumor by the method of embodiment 42, classifying the tumor by criteria comprising the relative expression of IR-A and IR-B.

44. A method of selecting a patient for treatment with an IGF antagonist comprising determining a relative expression of IR-A versus IR-B in a tissue of the patient by the method of embodiment 42, classifying the patient by criteria comprising the relative expression of IR-A and IR-B thereby predicating the relative responsiveness of the patient to an IGF antagonist.

45. The method of any of embodiments 39, 43 and 44, wherein the expression of IR-A is increased relative to IR-B.

46. A method of treating a patient using an IGF antagonist comprising determining a relative expression of IR-A versus IR-B in the tissue sample by the method of embodiment 42, classifying the patient by criteria comprising the relative expression of IR-A and IR-B; and, administering an IGF antagonist in accordance with the classification.

47. The method of embodiment 46, wherein the expression of IR-A is increased relative to IR-B.

48. The method of any of embodiments 44 or 47, wherein the IGF antagonist is an antibody.

49. The method of any of embodiments 40, 41, and 48, wherein the antibody comprises an amino acid sequence comprising the amino acid sequences of SEQ ID NOs: 45 and 53.

50. The method of any of embodiments 40, 41, and 48, wherein the antibody comprises the CDR sequences of SEQ ID NOs: 45 and 53, as shown in Tables 1 and 2.

51. The method of any of embodiments 40, 41, and 48, wherein, the antibody comprises three CDRs from SEQ ID NO: 45 and a light chain.

52. The method of any of embodiments 40, 41, and 48, wherein the antibody comprises three CDRs from SEQ ID NO: 53 and a heavy chain.

53. The method of any of embodiments 45 and 47, wherein the percentage of IR-A relative total insulin receptor is greater than 46.6%.

54. The method of any of embodiment 45 and 47, wherein the IR-A:IR-B $\Delta\Delta Ct$ is less than about 0.2.

55. The method of any of embodiment 45 and 47, wherein the mean relative quantity differentials on a log 2-base scale for IR-A is about -0.07 ± 0.29 is about -2.08 ± 0.25 for IR-B.

56. A method of classifying a breast cancer tumor subtype, the method comprising determining a relative expression of IR-A versus IR-B in a breast cancer tumor sample by the method of embodiment 42; and, classifying the breast cancer subtype as luminal B if the IR-A:IR-B ratio is lower than a threshold value.

57. The method of embodiment 56 further comprising calculating IR-A:IR-B $\Delta\Delta Ct$, wherein the threshold is a IR-A:IR-B $\Delta\Delta Ct$ value set in the range between about -1.1 to about -2.4.

58. The method of embodiment 54, wherein the threshold is in the range between about -1.4 to -2.1.

59. The method of embodiment 57, wherein a determination of IR-A:IR-B $\Delta\Delta Ct$ may be combined with other expression profiles in determining subtype classifications.

60. A method of determining a proliferation score for a tumor, the method comprising determining a relative expression of IR-A versus IR-B in a tumor sample by the method of embodiment 42, and considering a higher IR-A:IR-B ratio as a factor indicating a higher proliferation score.

61. The method of embodiment 60, further comprising calculating IR-A:IR-B $\Delta\Delta Ct$ and considering a lower IR-A:IR-B $\Delta\Delta Ct$ value as a factor indicating a higher proliferation score.

62. A method of classifying a breast cancer sample as luminal-A or luminal-B, comprising determining the relative levels of IR-A and IR-B in the sample, wherein an increased amount of IR-A relative to IR-B indicates a tumor that is luminal-B.

EXAMPLES

Example 1

IR-A and IR-B Primer and Probe Design

Commercially purchased assays were unable to distinguish and quantify IR-A and IR-B expression. Thus, novel probes were required. Mature mRNA transcript sequences for the insulin receptor (INSR) short (NM 001079817) and long (NM 000208) isoforms were obtained from the National Center for Biotechnology Information (NCBI) Entrez Nucleotide database. The INSR short isoform is designated as IR-A and the INSR long isoform as IR-B. The difference between the two isoforms is the presence (in IR-B) or absence (in IR-A) of exon 11, a 36 nucleotide region, in the mature transcript. Exon 11 is absent in the IR-A form, while the IR-B form contains the exon 11 sequence in the mature mRNA transcript. For the design of primers and probes that are specific for the detection of IR-A mRNA, the exon 10/12 junction region was targeted for the gene specific probe. Several primer pairs (forward and reverse) located within the exon 10 or exon 12 coding regions, respectively, were designed. For the IR-B design, the exon 11/12 junction was targeted and the exon 11 interior region for the gene specific probe. Several primer pairs (forward and reverse) located within exon 10 or on the exon 10/11 junction and exon 12 coding regions, respectively, were designed. All primer/probe designs were imported into the Primer Express (ABI) software tool to ensure optimal design for utilization in the TaqMan Gene Expression assay procedure. All probes were designed to incorporate a minor groove binding (MGB) moiety, and were labeled with a fluorescent dye (FAM) for detection and a non-fluorescent quencher. Sequences for all primer/probe combinations designed are presented Tables 3 and 4.

TABLE 3

Primer and Probes of IR-A assay. IR-A Primer Probe Designs:			
Name	Forward Primer (5'-->3')	Reverse Primer (5'-->3')	Probe (FAM-MGB) (5'-->3')
IRA	CAGGCCATCTCGG AAACG SEQ ID NO: 16	ACGGCCACCGTCAC ATTC SEQ ID NO: 20	AGGTCCTTGGCGATG SEQ ID NO: 24
IRA1	TGAGGATTACCTG CACACG SEQ ID NO: 3	ACCGTCACATTCCC AACATC SEQ ID NO: 21	TCCCCAGGCCATCT SEQ ID NO: 7
IRA2	CTGGTGCCGAGGA CCCTAGG SEQ ID NO: 17	ACCGTCACATTCCC AACATC SEQ ID NO: 21	TCCCCAGGCCATCT SEQ ID NO: 7
IRA3	TTGAGGATTACCT GCACACGT SEQ ID NO: 5	GCCAAGGGACCTGC GTTT SEQ ID NO: 22	TTTTCGTCCTCCAG GCCA SEQ ID NO: 8
IRA4	CTGCACACGTGG TTTTCGT SEQ ID NO: 18	CACCGTCACATTCC CAACATC SEQ ID NO: 23	CAGGCCATCTCGGAAA SEQ ID NO: 25

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TABLE 3-continued

Primer and Probes of IR-A assay. IR-A Primer Probe Designs:			
Name	Forward Primer (5'-->3')	Reverse Primer (5'-->3')	Probe (FAM-MGB) (5'-->3')
IRA5	TTGAGGATTACCT GCACACGT SEQ ID NO: 19	GCCAAGGGACCTGC GTTT SEQ ID NO: 22	TTCGTCCCCAGGCCA SEQ ID NO: 26

TABLE 4

Primer and Probes of IR-B assay. IR-B Primer Probe Designs:			
Name	Forward Primer (5'-->3')	Reverse Primer (5'-->3')	Probe (FAM-MGB) (5'-->3')
IRB	ACCTAGGCCATC TCGGAAA SEQ ID NO: 27	CACGGCCACCGTCA CATT SEQ ID NO: 28	CCTGGCGATGTTGG SEQ ID NO: 29
IRB1	TGAGGATTACCTG CACAAACG SEQ ID NO: 3	ACCGTCACATTCCC AACATC SEQ ID NO: 21	GAGGACCTAGGCCA SEQ ID NO: 30
IRB2	CTGGTGCCGAGGA CCCTAGG SEQ ID NO: 17	ACCGTCACATTCCC AACATC SEQ ID NO: 21	TGCCGAGGACCCTA SEQ ID NO: 31
IRB3	CGTCCCCAGAAAA ACCTCTTC SEQ ID NO: 11	GGACCTGCGTTTCC GAGAT SEQ ID NO: 12	ACTGGTGCCGAGGAC SEQ ID NO: 13
IRB4	CGTCCCCAGAAAA ACCTCTTC SEQ ID NO: 11	GGACCTGCGTTTCC GAGAT SEQ ID NO: 12	CCGAGGACCTAGGC SEQ ID NO: 14
IRB5	CGTCCCCAGAAAA ACCTCTTC SEQ ID NO: 11	GGACCTGCGTTTCC GAGAT SEQ ID NO: 12	TGCCGAGGACCCTAG SEQ ID NO: 15

Example 2

Specificity of the Primers and Probes

Commercially available plasmids containing full length cDNA clones for the INSR long (clone SC311328) and short (clone SC315880) transcripts were purchased from OriGene Technologies, Inc. Sequence verification of each INSR clone was conducted. All TaqMan Gene Expression assay designs were tested for specificity and sensitivity in the presence of either the INSR long or short isoform clones at various copy number inputs (102-107 copies). Standard TaqMan Gene Expression assays were conducted in a 384-well format for all primer/probe and template combinations. Reactions consisted of 7.5 μ L of TaqMan Universal Master Mix, 1.5 μ L of 10 \times Gene Expression Assay Mix, and 6 μ L of varying copy numbers of either the INSR long or short form cDNA clone, for a final volume of 15 μ L per well of a 384-well plate. Each primer/probe and template combination was repeated at least 3 times. All assay plates were run on an Applied Biosystems 7900HT detection system using standard settings (cycling program consisting of a 10 min incubation at 95 C followed by 40 cycles of 95 $^{\circ}$ C. for 15 sec and 60 $^{\circ}$ C. for 1 min). Data values (Ct values) were extracted from each assay run with the SDS v2.0 software tool (ABI). Following data extraction and analysis, it was determined that the following primer and probe assay designs provided sufficient sensitivity and specificity for our purposes; IR-A1, IR-A3, IR-B3, IR-B4, and IR-B5.

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The IR-A1 and IR-A3 assays were able to detect the IR-A isoform sequence at a copy number threshold of about 35 copies. These assays were also determined to be highly specific for IR-A isoform since they were unable to detect the presence of IR-B isoform at any copy number input utilized. Alternatively, the IR-B3, IR-B4, and IR-B5 designs were able to detect IR-B isoform sequence at a copy number threshold of about 35 copies. These designs were also determined to be specific for IR-B isoform since they were unable to detect the presence of IR-A isoform below a threshold of about 35 copies and they were unable to detect the presence of IR-B isoform at any copy number input utilized.

These specificity experiments were repeated utilizing the BioMarkTM Dynamic Array (Fluidigm Corporation) microfluidics system for Real-Time PCR. This system allows for high throughput real-time PCR (2304 individual reactions possible per plate), producing high quality data with low variability and a tight correlation with conventional RT-PCR. To employ this technology, cDNA samples were pre-amplified using TaqMan Pre-Amp Master Mix, according to the manufacturer's instructions. Reactions contained 5 μ L of cDNA, 10 μ L Pre-Amp Master Mix, and 5 μ L of 0.2 \times gene expression assay mix (comprised of all primer/probes to be assayed) for a final volume of 20 μ L. Reactions were cycled with the recommended program for 14 cycles and then diluted 1:5 with TE buffer. Pre-amplified cDNA was either utilized immediately or stored at -20 C until needed.

To prepare samples for loading into 48 \times 48 dynamic array chips (Fluidigm), the reaction mix contained 2.5 μ L 2 \times Universal Master Mix (Applied Biosystems), 0.25 μ L Sample Loading Buffer (Fluidigm Corporation), and 2.25 μ L pre-amplified cDNA. To prepare the primer/probes, the reaction mix contained 2.5 μ L 20 \times Taqman Gene Expression Assay and 2.5 μ L Assay Loading Buffer (Fluidigm Corporation). Prior to loading the samples and assay reagents into the inlets, the chip was primed in the IFC Controller. Five μ L of sample prepared as described was loaded into each sample inlet of the dynamic array chip and 5 μ L of 10 \times gene expression assay mix was loaded into each detector inlet. The chip was placed on the IFC Controller for loading and mixing. After approximately 1 hr, the chip was loaded on the BioMarkTM Real-Time PCR System for thermal cycling (10 min at 95 $^{\circ}$ C. followed by 40 cycles of 95 $^{\circ}$ C. for 15 sec and 1 min at 60 $^{\circ}$ C.). The number of replicates and the composition of the samples varied depending on the particular experiment, but were never less than triplicate. Average Ct values were used to determine sensitivity and specificity of the designed probes.

The results obtained using conventional real-time PCR were confirmed using the Fluidigm system. IR-A1, IR-A3, IR-B3, IR-B4, and IR-B5 were specific for either IR-A or IR-B and were able to detect the appropriate receptor isoform at a copy number threshold of about 35 copies.

Additional analyses also utilized cDNA template from cell lines either known to over-express either IR-A or IR-B or engineered to over-express IR-A. Delta comparative threshold (Δ Ct) values for each sample were calculated by subtracting the average CT of the 2 endogenous control genes (GAPDH and ACTIN) from the average Ct of the target gene. Results indicated that these primer/probe designs reproducibly and specifically detected either IR-A or IR-B in cell lines in the same manner observed using cDNA clones. Together, these results validate the use of Fluidigm technology for further high throughput analysis of cDNA or tissue samples, as well as confirming the specificity of the IR-A and IR-B primer/probe designs. Following qualification of multiple

primer/probe designs, we selected IR-A1 and IR-B4 to measure the expression status of IR-A and IR-B in a large set of breast cancers.

Probe sensitivity was confirmed by performing an IR-A or IR-B qRT-PCR assay starting with 100 pg template stock solutions of IR-A or IR-B (approximately 10^7 copies of DNA template). The DNA was serially diluted to 10^{-4} pg (approximately 10 copies DNA template). Each sample was tested in duplicate. The slope of the standard template dilution curve was determined by plotting cycle-threshold (Ct) values as a function of the log DNA copy number. The results are shown in FIG. 7. Strong correlations were observed between the log [concentration] and resultant Ct values for each assay tested with its respective matching standard template. All correlation coefficients (r^2 value) were ≥ 0.999 ($p \leq 0.0001$). Linearities were maintained in the DNA concentration ranges described above in both assays, demonstrating a wide dynamic range and yielding accurate Ct values. The results indicated that both the IR-A and IR-B assays are sensitively detecting the appropriate isoform to ~ 35 copies of DNA.

The specificities of the assays were also assessed by testing the IR-A assay in the presence of the IR-B DNA template or the IR-B assay in presence of the IR-A DNA template, respectively. The IR-A assay does not amplify IR-B DNA template in the tested range of 10 to 10^7 copies of IR-B DNA. Likewise, the IR-B assay does not amplify the IR-A DNA template in the range tested.

The assay efficiency was assessed by the slopes of the standard dilution curves for both assays (FIG. 7). The slope is -3.259 for IR-A and -3.155 for IR-B. The two slopes are very similar, suggesting little differences in probe efficiency.

Example 3

Expression Profiling in Breast Cancer Patients

A. General Methodology

Forty-two grade I to III infiltrating breast ductal carcinomas were purchased from ILSbio (Chestertown, Md.). 19 matched normal adjacent breast tissue samples were also procured. The ages of patients ranged from 31 to 88 years. All the breast cancer samples are ER and PR positive and HER2 negative according IHC. All samples were fresh frozen and collected before initiation of any treatment. Tumor samples were macrodissected to remove normal tissue and the normal samples were macrodissected to remove non-glandular tissue. After macrodissection, the tumor purity in all samples is greater than 85%.

Four breast cancer tissue qPCR cDNA arrays (BCRT101, BCRT102, BCRT103, BCRT104) were purchased from OriGene Technologies (Rockville, Md.). The qPCR arrays contain cDNAs from 15 normal breast tissues (from 10 unique donors) and 165 breast adenocarcinoma tissues. The tumor stage varied from stage I to IV and the tissues were comprised of 50-90% tumor.

Total RNA was extracted from snap-frozen tissue specimens using the ZR RNA MicroPrep kit (Zymo Research, Orange, Calif.). RNA purity and concentration were determined spectrophotometrically ($260/280 > 1.9$). RNA quality was assessed on an Agilent 2100 Bioanalyzer using the RNA 6000 Nano LabChip®.

For the following examples, the sequence of the forward primer for IR-A assay was 5'-TGAGGATTACCTGCA-CAACG-3' (SEQ ID NO: 3), and the sequence of the reverse primer is 5'-ACCGTCACATTCCCAACATC-3' (complement of SEQ ID NO:4), and the probe 5'-TCCCCAGGC-CATCT-3' (SEQ ID NO:7). The sequence of the forward

primer for the IR-B assay was 5'-CGTCCCCAGAAAAAC-CTCTTC-3' (SEQ ID NO:11), and the sequence of reverse primer is 5'-GGACCTGCGTTTCCGAGAT-3' (SEQ ID NO:12), and the sequence of the probe is 5' CCGAGGAC-CCTAGGC-3' (SEQ ID NO:14).

For positive and negative controls, commercially available cDNA clones which contain the full-length cDNA clone of IR-A (cloned in pCMV6-XL4) and IR-B (cloned in pCMV6-XL5) were purchased from OriGene Technologies, Inc (IR-A: SKU#. SC311328; IR-B: SKU# SC315880). The empty plasmids of pCMV6-XL4 and pCMV6-XL5 were used as negative control DNA for IR-A and IR-B assays, respectively.

Standard TaqMan Gene Expression assays were conducted in a 384-well format for all primer/probe and template combinations. Reactions consisted of 5 μ L of TaqMan Universal Master Mix, 0.5 μ L of 10 \times Gene Expression Assay Mix, and 4.5 μ L of varying copy numbers of either the IR-B or IR-A cDNA clone, for a final volume of 10 μ L per well of a 384-well plate. Each primer/probe and template combination was repeated at least 3 times. All assay plates were run on an Applied Biosystems 7900HT detection system using standard settings (cycling program consisting of a 10 min incubation at 95° C. followed by 40 cycles of 95° C. for 15 sec and 60° C. for 1 min). Data values (Cycle Threshold (Ct) values) were extracted from each assay run with the SDS v2.0 software tool (ABI).

For assessment of the expression levels of other genes, TaqMan Gene Expression assays were purchased from ABI (Forest city, Calif.) The assays include: INSR (Assay ID: Hs00961554_m1), ER (Assay ID: Hs00174860_m1), PR (Assay ID: Hs01556707_m1), ERBB2 (HER2, Assay ID: Hs01001580_m1), tumor proliferation genes (Pike S et al 2004): BIRC5 (Assay ID: Hs00153353_m1), AURKA (STK15, Assay ID: Hs01582073_m1), CCNB1 (Assay ID: Hs00259126_m1), Ki67 (Assay ID: Hs01032443_m1), MYBL2 (Assay ID: Hs00942543_m1) and reference or "housekeeping" genes: ACTB (Hs99999903_m1), GUSB (Assay ID: Hs99999908_m1), GAPDH (Assay ID: Hs99999905_m1), RPLPO (Assay ID: Hs99999902_m1), TFRC (Assay ID: Hs99999911_m1).

BioMark™ Dynamic Array (Fluidigm Corporation) microfluidics system allows for high throughput real-time PCR (2304 individual reactions possible per plate), producing high quality data with low variability and a tight correlation with conventional RT-PCR. Single stranded cDNA was generated from total RNA using the SuperScript® III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, Calif.). cDNA samples were pre-amplified using TaqMan Pre-Amp Master Mix, according to the manufacturer's instructions. Reactions contained 5 μ L of cDNA, 10 μ L Pre-Amp Master Mix, and 5 μ L of 0.2 \times gene expression assay mix (comprised of all primer/probes to be assayed) for a final volume of 20 μ L. Reactions were cycled with the recommended program for 14 cycles and then diluted 1:5 with TE buffer. Pre-amplified cDNA was either utilized immediately or stored at -20° C. until needed.

To prepare samples for loading into 48-48 dynamic array chips (Fluidigm), the reaction mix contained 2.5 μ L 2 \times Universal Master Mix (Applied Biosystems), 0.25 μ L Sample Loading Buffer (Fluidigm Corporation), and 2.25 μ L pre-amplified cDNA. To prepare the primer/probes, the reaction mix contained 2.5 μ L 20 \times Taqman Gene Expression Assay and 2.5 μ L Assay Loading Buffer (Fluidigm Corporation). Prior to loading the samples and assay reagents into the inlets, the chip was primed in the IFC Controller. Five μ L of sample prepared as described was loaded into each sample inlet of the dynamic array chip and 5 μ L of 10 \times gene expression assay

mix was loaded into each detector inlet. The chip was placed on the IFC Controller for loading and mixing. After approximately 1 hr, the chip was loaded on the BioMark™ Real-Time PCR System for thermal cycling (10 min at 95° C. followed by 40 cycles of 95° C. for 15 sec and 1 min at 60° C.). The number of replicates and the composition of the samples varied depending on the particular experiment, but were never less than triplicate. Average Ct values were used to determine sensitivity and specificity of the designed probes. The average Ct values of all available reference gene assays, within a sample, were used for Δ Ct calculation.

Generation of biotin-labeled amplified cRNA from 75 ng of total RNA was accomplished with the MessageAmp™ Premier RNA Amplification Kit (Ambion, Austin, Tex.). The concentration and purity of the cRNA product were determined spectrophotometrically. Fifteen micrograms of each biotin-labeled cRNA was fragmented for hybridization on Affymetrix Human Genome U133 Plus 2.0 GeneChip® arrays. All GeneChip® washing, staining, and scanning procedures were performed with Affymetrix standard equipment. Data capture and initial array quality assessments were performed with the GeneChip Operating Software (GCOS) tool. Any probe displaying a signal intensity <25 across all samples was excluded from the analysis.

A subset of ER+, PR+ and Her2- primary breast tumors (n=40) and matched normal adjacent breast tissue samples (n=15) was profiled on Affymetrix Human Genome U133 Plus 2.0 GeneChip® arrays. Two of the primary breast samples and four of the matched normal adjacent breast tissue samples analyzed on the Fluidigm platform were not processed on GeneChip due to insufficient RNA quantity. Breast cancer molecular subtype classification, with regards to luminal-A and luminal-B subtype, was conducted utilizing our whole genome array data.

Two methods for determining putative sample classification were implemented. The first classification method utilized a published PAM50-gene shrunken centroid classifier (Weigelt, et al. 2010) for sample sub-typing (normal, basal-like, HER2, luminal-A, or luminal-B) purposes. MASS normalized GeneChip data was used for this analysis given that the published classifier was built using this type of scaled data. The samples were classified according to a Spearman's rank correlation (50-gene intensity vector vs. subtype centroid classifier), where the subtype with the highest correlation value was assigned to a particular sample. The second method utilized GC-RMA normalized GeneChip data to identify a panel of differentially expressed transcripts by a two-sample Welch's t-test analysis. Samples were divided into two groups (normal or tumor) based on pathology assessment prior to conducting the statistical analysis. Probes displaying a fold change differential >3 and p-value <1.0×10⁻¹² (n=459 probes) were used for an unsupervised hierarchical clustering analysis. Sub-populations identified were classified as normal, luminal-A, or luminal-B as a function of transcript panel composition.

B. Results

IR-A and IR-B in Breast Cancer

The mRNA expression status of IR-A and IR-B in breast cancer, 42 ER and PR positive and Her2 negative primary breast tissue samples and 19 matched normal adjacent breast tissues were studied. Random hexamer primed cDNAs were pre-amplified and assayed for expression levels of IR-A, IR-B, and total insulin receptor (INSR) transcripts by TaqMan qPCR (Fluidigm). Samples were normalized to the average of five housekeeping genes as described above. The results are shown in FIG. 8. The mean relative quantity (RQ) differentials (log_e-base scale)±95% CI of INSR, IR-A, and

IR-B in normal (n=19) were 1.03×10⁻⁸±0.17, -7.37×10⁻⁹±0.24, and 3.37×10⁻⁸±0.18, respectively. The mean relative quantity (RQ) differentials (log 2-base scale) ±95% CI of INSR, IR-A, and IR-B in tumor (n=42) were -0.88±0.25, -0.07±0.29, and -2.08±0.25, respectively. A two-tailed Welch's t-test analysis indicate that the levels of mRNA of INSR and IR-B are significantly lower in the tested breast tumor set when compared to the normal breast tissue (p<0.0001). Alternatively, no significant differences were observed in the mRNA levels of IR-A in breast cancer when compared with normal (p=0.4501).

The proportion of IR-A relative to total insulin receptor composition (i.e. IR-A+IR-B) in matched tumor and normal pairs was calculated by 2^(-ΔCt). The results are shown in FIG. 9. The mean IR-A transcript proportion (%)±95% CI for the normal panel (n=19) was 46.60%±4.74%, while the mean IR-A transcript proportion (%)±95% CI for the matched tumor samples was 75.24%±5.02. A paired sample t-test analysis indicated that a significant increase of the calculated IR-A proportion in tumor samples exists when compared to matched normals (p<0.0001). Results suggest that the significantly decreased IR-B levels in tumor contribute to an overall increase the proportion of IR-A in tumor samples compared to normal.

In order to assess the mRNA transcript ratios of IR-A and IR-B, we calculated ΔCt differentials of IR-A and IR-B in normal and primary tumor breast samples. The ΔCt differentials (IR-A ΔCt-IR-B ΔCt) values were calculated for all samples utilizing the within-sample reference gene (house-keeping) panel (average Ct) for normalization purposes. The mean IR-A:IR-B ΔCt±95% CI was 0.20±0.23 for normal (n=19) and the mean IR-A:IR-B ΔCt±95% CI was -1.81±0.27 in primary tumors (n=42). A two-tailed Welch's t-test analysis identified a significant difference between normal and tumor samples in relation to observed IR-A:IR-B ΔCt (p<0.0001) (FIG. 10). The results indicated a significant increased ratio of IR-A to IR-B in breast tumors.

To further validate the above results, we assessed mRNA expression ratios of IR-A and IR-B in more breast cancer tissue samples. PCR arrays containing cDNAs from 15 normal breast tissues and 165 breast adenocarcinoma tissues were used. Equal amounts of cDNA were pre-amplified and assayed for expression levels of IR-A, IR-B, and ER by TaqMan qPCR (Fluidigm). The ΔCt differentials (IR-A ΔCt-IR-B ΔCt) values were calculated for all samples utilizing the within-sample reference gene panel (ACTB, GUSB, GAPDH) for normalization purposes. The results are shown in FIG. 11. The mean IR-A:IR-B ΔCt±95% CI was 0.51±0.37 in normal tissues (n=15). The mean IR-A:IR-B ΔCt±95% CI was -1.19±0.17 across all breast cancers examined (n=165).

We then separated the breast cancer samples into those that displayed an estrogen receptor over-expression of 2-fold relative to normal breast tissue and compared their IR-A:IR-B ΔCt differentials to normal tissue and to all breast cancer samples. The results are shown in FIG. 11. The mean IR-A:IR-B ΔCt±95% CI was -1.48±0.39 in ER+ breast cancers (n=83), which is very similar to that observed across the whole breast cancer dataset. A two-tailed Welch's t-test analysis identified a significant difference between normal and tumor samples in relation to observed IR-A:IR-B ΔCt differential (p<0.0001).

Correlating IR-A: IR-B Ratio with Genes Involved in Breast Cancer Proliferation

Ki67, STK15, Survivin, CCNB1, MYBL2 are well characterized genes involved in breast cancer proliferation. The composite expression score of these genes has been used in Oncotype DX and is an important factor contributing to the

breast cancer recurrence in many patients. We studied the relationship of the IR-A:IR-B ratio and the proliferation score in the primary breast cancer sample sets using regression and correlation analyses. Linear regression analysis was conducted to quantify the relationship between the calculated IR-A:IR-B Δ Ct differential and a pooled panel of proliferation markers (AURKA, BIRC5, CCNB1, KI67, and MYBL2). Proliferation panel summary values were calculated by taking the average Δ Ct across all markers for a particular sample. Summary results for both normal and tumor samples are presented. The linear regression analysis results suggest a positive correlation between the two summary values (adjusted $r^2=0.595$) (FIG. 12). The IR-A:IR-B Δ Ct differential exhibits a positive correlation with the proliferation score (FIG. 12). The results suggest that the decreasing IR-B expression in tumor and increasing IR-A proportion expression may contribute tumor proliferation in ER+ PR+ and Her- breast cancer.

IR-A:IR-B Δ Ct Differential in Breast Cancer Subtypes.

Breast cancer is a heterogeneous disease with respect to molecular alterations, cellular composition, and clinical outcome. Using an intrinsic gene list, ER positive breast cancers can be further classified by hierarchical cluster analysis into luminal-A, and luminal-B subtypes (Perou C M, 2000). Luminal-A cancers are histologically low-grade and sensitive to neo-adjuvant endocrine therapy (Creighton C et al 2008). In contrast, luminal-B cancers are often histologically high-grade and less sensitive to neo-adjuvant endocrine therapy, with a shorter time to poor outcome events (Creighton C et al 2008). Creighton reported that an IGF-I signature is manifested in luminal-B breast cancers and this signature is both highly correlated with numerous poor prognostic factors and one of the strongest indicators of disease outcome. Since the IR-A isoform is one of the important components involved the IGF signaling, we investigated the hypothesis that alterations in IR-A:IR-B ratios may be evident when comparing luminal-A and luminal-B breast cancers.

To address this question we conducted whole genome array analysis on 40 ER+ PR+ and Her2- negative breast tumor and 15 normal breast samples. We initially utilized a published PAM50-gene shrunken centroid classifier (Weigelt, et al. 2010). Samples were classified as luminal A or luminal B according to a Spearman's rank correlation, where the subtype with the highest correlation value was assigned to a particular sample. IR-A:IR-B Δ Ct differentials in the normal, luminal-A and luminal-B were then compared. The scatter plot representation of calculated IR-A:IR-B Δ Ct differentials with regards to sample subtype (normal, luminal-A, or luminal-B) are shown in FIG. 13A. The mean IR-A:IR-B Δ Ct \pm 95% CI was 0.27 ± 0.30 in normal (n=15). The mean IR-A:IR-B Δ Ct \pm 95% CI was -1.09 ± 0.34 in luminal-A classified breast cancers (n=13). The mean IR-A:IR-B Δ Ct \pm 95% CI was -2.12 ± 0.34 in luminal-B classified breast cancers (n=27). All subtype pair-wise comparisons display a significant difference (two-sample t-test, $p<0.001$). The results indicated that IR-A:IR-B ratios in luminal-B patients changed more drastically than luminal-A patients.

In addition to the shrunken centroid classifier, we utilized GC-RMA normalized GeneChip data to identify a panel of differentially expressed transcripts by a two-sample Welch's t-test analysis. Samples were divided into two groups (normal or tumor) based on pathology assessment prior to conducting the statistical analysis. Sub-populations identified by unsupervised hierarchical clustering were classified as normal, luminal-A, or luminal-B as a function of transcript panel composition. IR-A:IR-B Δ Ct differentials in the normal, luminal-A and luminal-B were also compared. The results are

shown in FIG. 13B. The mean IR-A:IR-B Δ Ct \pm 95% CI was 0.32 ± 0.25 in normal (n=15). The mean IR-A:IR-B Δ Ct \pm 95% CI was -1.05 ± 0.19 in luminal-A predicted breast cancers (n=18). The mean IR-A:IR-B Δ Ct \pm 95% CI was -2.42 ± 0.32 in luminal-B predicted breast cancers (n=22). All subtype pair-wise comparisons display a significant difference (two-sample t-test, $p<0.001$).

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While the disclosure above has been provided in detail with reference to preferred aspects thereof, it will be apparent to

one skilled in the art that various changes can be made, and equivalents employed, without departing from the scope of

the disclosure and the claims. All documents cited are incorporated by reference in their entireties.

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Gly	Gln	Pro	Thr	Leu	Val	Val	Met	Glu	Leu	Met	Ala	His	Gly	Asp		
				1085					1090					1095		
Leu	Lys	Ser	Tyr	Leu	Arg	Ser	Leu	Arg	Pro	Glu	Ala	Glu	Asn	Asn		
				1100					1105					1110		
Pro	Gly	Arg	Pro	Pro	Pro	Thr	Leu	Gln	Glu	Met	Ile	Gln	Met	Ala		
				1115					1120					1125		
Ala	Glu	Ile	Ala	Asp	Gly	Met	Ala	Tyr	Leu	Asn	Ala	Lys	Lys	Phe		
				1130					1135					1140		
Val	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	Cys	Met	Val	Ala	His	Asp		
				1145					1150					1155		
Phe	Thr	Val	Lys	Ile	Gly	Asp	Phe	Gly	Met	Thr	Arg	Asp	Ile	Tyr		
				1160					1165					1170		
Glu	Thr	Asp	Tyr	Tyr	Arg	Lys	Gly	Gly	Lys	Gly	Leu	Leu	Pro	Val		
				1175					1180					1185		
Arg	Trp	Met	Ala	Pro	Glu	Ser	Leu	Lys	Asp	Gly	Val	Phe				

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Ser	Ser	Asp	Met	Trp	Ser	Phe	Gly	Val	Val	Leu	Trp	Glu	Ile	Thr
1205						1210					1215			
Ser	Leu	Ala	Glu	Gln	Pro	Tyr	Gln	Gly	Leu	Ser	Asn	Glu	Gln	Val
1220						1225					1230			
Leu	Lys	Phe	Val	Met	Asp	Gly	Gly	Tyr	Leu	Asp	Gln	Pro	Asp	Asn
1235						1240					1245			
Cys	Pro	Glu	Arg	Val	Thr	Asp	Leu	Met	Arg	Met	Cys	Trp	Gln	Phe
1250						1255					1260			
Asn	Pro	Lys	Met	Arg	Pro	Thr	Phe	Leu	Glu	Ile	Val	Asn	Leu	Leu
1265						1270					1275			
Lys	Asp	Asp	Leu	His	Pro	Ser	Phe	Pro	Glu	Val	Ser	Phe	Phe	His
1280						1285					1290			
Ser	Glu	Glu	Asn	Lys	Ala	Pro	Glu	Ser	Glu	Glu	Leu	Glu	Met	Glu
1295						1300					1305			
Phe	Glu	Asp	Met	Glu	Asn	Val	Pro	Leu	Asp	Arg	Ser	Ser	His	Cys
1310						1315					1320			
Gln	Arg	Glu	Glu	Ala	Gly	Gly	Arg	Asp	Gly	Gly	Ser	Ser	Leu	Gly
1325						1330					1335			
Phe	Lys	Arg	Ser	Tyr	Glu	Glu	His	Ile	Pro	Tyr	Thr	His	Met	Asn
1340						1345					1350			
Gly	Gly	Lys	Lys	Asn	Gly	Arg	Ile	Leu	Thr	Leu	Pro	Arg	Ser	Asn
1355						1360					1365			
Pro	Ser													
1370														

<210> SEQ ID NO 33

<211> LENGTH: 1382

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

Met	Gly	Thr	Gly	Arg	Arg	Gly	Ala	Ala	Ala	Ala	Pro	Leu	Leu	Val
1			5				10					15		
Ala	Val	Ala	Ala	Leu	Leu	Leu	Gly	Ala	Ala	Gly	His	Leu	Tyr	Pro
	20						25					30		
Glu	Val	Cys	Pro	Gly	Met	Asp	Ile	Arg	Asn	Asn	Leu	Thr	Arg	Leu
	35					40					45			
Glu	Leu	Glu	Asn	Cys	Ser	Val	Ile	Glu	Gly	His	Leu	Gln	Ile	Leu
	50				55						60			
Met	Phe	Lys	Thr	Arg	Pro	Glu	Asp	Phe	Arg	Asp	Leu	Ser	Phe	Pro
65				70					75				80	
Leu	Ile	Met	Ile	Thr	Asp	Tyr	Leu	Leu	Leu	Phe	Arg	Val	Tyr	Gly
		85					90						95	
Glu	Ser	Leu	Lys	Asp	Leu	Phe	Pro	Asn	Leu	Thr	Val	Ile	Arg	Gly
		100					105						110	
Arg	Leu	Phe	Phe	Asn	Tyr	Ala	Leu	Val	Ile	Phe	Glu	Met	Val	His
	115					120					125			
Lys	Glu	Leu	Gly	Leu	Tyr	Asn	Leu	Met	Asn	Ile	Thr	Arg	Gly	Ser
	130					135					140			
Arg	Ile	Glu	Lys	Asn	Asn	Glu	Leu	Cys	Tyr	Leu	Ala	Thr	Ile	Asp
145				150					155				160	
Ser	Arg	Ile	Leu	Asp	Ser	Val	Glu	Asp	Asn	Tyr	Ile	Val	Leu	Asn
		165					170						175	
Asp	Asp	Asn	Glu	Glu	Cys	Gly	Asp	Ile	Cys	Pro	Gly	Thr	Ala	Lys
		180					185						190	

Lys	Thr	Asn	Cys	Pro	Ala	Thr	Val	Ile	Asn	Gly	Gln	Phe	Val	Glu	Arg
		195					200					205			
Cys	Trp	Thr	His	Ser	His	Cys	Gln	Lys	Val	Cys	Pro	Thr	Ile	Cys	Lys
	210					215					220				
Ser	His	Gly	Cys	Thr	Ala	Glu	Gly	Leu	Cys	Cys	His	Ser	Glu	Cys	Leu
225					230					235					240
Gly	Asn	Cys	Ser	Gln	Pro	Asp	Asp	Pro	Thr	Lys	Cys	Val	Ala	Cys	Arg
				245					250					255	
Asn	Phe	Tyr	Leu	Asp	Gly	Arg	Cys	Val	Glu	Thr	Cys	Pro	Pro	Pro	Tyr
			260					265					270		
Tyr	His	Phe	Gln	Asp	Trp	Arg	Cys	Val	Asn	Phe	Ser	Phe	Cys	Gln	Asp
		275					280					285			
Leu	His	His	Lys	Cys	Lys	Asn	Ser	Arg	Arg	Gln	Gly	Cys	His	Gln	Tyr
	290					295					300				
Val	Ile	His	Asn	Asn	Lys	Cys	Ile	Pro	Glu	Cys	Pro	Ser	Gly	Tyr	Thr
305					310					315					320
Met	Asn	Ser	Ser	Asn	Leu	Leu	Cys	Thr	Pro	Cys	Leu	Gly	Pro	Cys	Pro
				325					330					335	
Lys	Val	Cys	His	Leu	Leu	Glu	Gly	Glu	Lys	Thr	Ile	Asp	Ser	Val	Thr
			340					345					350		
Ser	Ala	Gln	Glu	Leu	Arg	Gly	Cys	Thr	Val	Ile	Asn	Gly	Ser	Leu	Ile
	355						360					365			
Ile	Asn	Ile	Arg	Gly	Gly	Asn	Asn	Leu	Ala	Ala	Glu	Leu	Glu	Ala	Asn
	370					375					380				
Leu	Gly	Leu	Ile	Glu	Glu	Ile	Ser	Gly	Tyr	Leu	Lys	Ile	Arg	Arg	Ser
385					390					395					400
Tyr	Ala	Leu	Val	Ser	Leu	Ser	Phe	Phe	Arg	Lys	Leu	Arg	Leu	Ile	Arg
				405					410					415	
Gly	Glu	Thr	Leu	Glu	Ile	Gly	Asn	Tyr	Ser	Phe	Tyr	Ala	Leu	Asp	Asn
			420					425					430		
Gln	Asn	Leu	Arg	Gln	Leu	Trp	Asp	Trp	Ser	Lys	His	Asn	Leu	Thr	Ile
			435				440					445			
Thr	Gln	Gly	Lys	Leu	Phe	Phe	His	Tyr	Asn	Pro	Lys	Leu	Cys	Leu	Ser
	450					455					460				
Glu	Ile	His	Lys	Met	Glu	Glu	Val	Ser	Gly	Thr	Lys	Gly	Arg	Gln	Glu
465					470					475					480
Arg	Asn	Asp	Ile	Ala	Leu	Lys	Thr	Asn	Gly	Asp	Gln	Ala	Ser	Cys	Glu
				485					490					495	
Asn	Glu	Leu	Leu	Lys	Phe	Ser	Tyr	Ile	Arg	Thr	Ser	Phe	Asp	Lys	Ile
			500					505					510		
Leu	Leu	Arg	Trp	Glu	Pro	Tyr	Trp	Pro	Pro	Asp	Phe	Arg	Asp	Leu	Leu
		515					520					525			
Gly	Phe	Met	Leu	Phe	Tyr	Lys	Glu	Ala	Pro	Tyr	Gln	Asn	Val	Thr	Glu
	530					535					540				
Phe	Asp	Gly	Gln												

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Lys 610	Ser	Asp	Ile	Ile	Tyr	Val 615	Gln	Thr	Asp	Ala	Thr 620	Asn	Pro	Ser	Val
Pro 625	Leu	Asp	Pro	Ile	Ser 630	Val	Ser	Asn	Ser	Ser 635	Gln	Ile	Ile	Leu 640	
Lys	Trp	Lys	Pro	Pro	Ser 645	Asp	Pro	Asn	Gly	Asn 650	Ile	Thr	His	Tyr	Leu 655
Val	Phe	Trp	Glu	Arg	Gln	Ala	Glu	Asp	Ser	Glu 665	Leu	Phe	Glu	Leu	Asp 670
Tyr	Cys	Leu 675	Lys	Gly	Leu	Lys 680	Leu	Pro	Ser	Arg	Thr 685	Trp	Ser	Pro	Pro
Phe 690	Glu	Ser	Glu	Asp	Ser	Gln 695	Lys	His	Asn	Gln	Ser 700	Glu	Tyr	Glu	Asp
Ser 705	Ala	Gly	Glu	Cys	Cys 710	Ser	Cys	Pro	Lys	Thr 715	Asp	Ser	Gln	Ile	Leu 720
Lys	Glu	Leu	Glu	Glu	Ser 725	Ser	Phe	Arg	Lys	Thr 730	Phe	Glu	Asp	Tyr	Leu 735
His	Asn	Val	Val	Phe	Val 740	Pro	Arg	Lys	Thr 745	Ser	Ser	Gly	Thr	Gly	Ala 750
Glu	Asp	Pro 755	Arg	Pro	Ser	Arg 760	Lys	Arg	Arg	Ser	Leu 765	Gly	Asp	Val	Gly
Asn 770	Val	Thr	Val	Ala	Val 775	Pro	Thr	Val	Ala	Ala	Phe 780	Pro	Asn	Thr	Ser
Ser 785	Thr	Ser	Val	Pro	Thr 790	Ser	Pro	Glu	Glu	His 795	Arg	Pro	Phe	Glu	Lys 800
Val	Val	Asn	Lys	Glu 805	Ser	Leu	Val	Ile	Ser 810	Gly	Leu	Arg	His	Phe	Thr 815
Gly	Tyr	Arg	Ile	Glu 820	Leu	Gln	Ala	Cys	Asn 825	Gln	Asp	Thr	Pro	Glu	Glu 830
Arg 835	Cys	Ser	Val	Ala	Ala 840	Tyr	Val	Ser	Ala	Arg	Thr 845	Met	Pro	Glu	Ala
Lys 850	Ala	Asp	Asp	Ile	Val 855	Gly	Pro	Val	Thr	His 860	Glu	Ile	Phe	Glu	Asn
Asn 865	Val	Val	His	Leu	Met 870	Trp	Gln	Glu	Pro	Lys 875	Glu	Pro	Asn	Gly	Leu 880
Ile	Val	Leu	Tyr	Glu 885	Val	Ser	Tyr	Arg	Arg 890	Tyr	Gly	Asp	Glu	Glu	Leu 895
His	Leu	Cys	Val	Ser 900	Arg	Lys	His	Phe 905	Ala	Leu	Glu	Arg	Gly	Cys	Arg 910
Leu	Arg	Gly 915	Leu	Ser	Pro	Gly 920	Asn	Tyr	Ser	Val	Arg 925	Ile	Arg	Ala	Thr
Ser 930	Leu	Ala	Gly	Asn	Gly 935	Ser	Trp	Thr	Glu	Pro	Thr 940	Tyr	Phe	Tyr	Val
Thr 945	Asp	Tyr	Leu	Asp	Val 950	Pro	Ser	Asn	Ile	Ala 955	Lys	Ile	Ile	Ile	Gly 960
Pro	Leu	Ile	Phe	Val 965	Phe	Leu	Phe	Ser	Val	Val 970	Ile	Gly	Ser	Ile	Tyr 975
Leu	Phe	Leu	Arg	Lys 980	Arg	Gln	Pro	Asp 985	Gly	Pro	Leu	Gly	Pro	Leu	Tyr 990
Ala	Ser	Ser	Asn	Pro	Glu	Tyr 995	Leu	Ser	Ala	Ser	Asp 1000	Val	Phe	Pro	Cys 1005
Ser 1010	Val	Tyr	Val	Pro	Asp	Glu 1015	Trp	Glu	Val	Ser	Arg 1020	Glu	Lys	Ile	
Thr	Leu	Leu	Arg	Glu	Leu	Gly	Gln	Gly	Ser	Phe	Gly	Met	Val	Tyr	

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1025	1030	1035
Glu Gly Asn Ala Arg Asp Ile	Ile Lys Gly Glu Ala	Glu Thr Arg
1040	1045	1050
Val Ala Val Lys Thr Val Asn	Glu Ser Ala Ser Leu	Arg Glu Arg
1055	1060	1065
Ile Glu Phe Leu Asn Glu Ala	Ser Val Met Lys Gly	Phe Thr Cys
1070	1075	1080
His His Val Val Arg Leu Leu	Gly Val Val Ser Lys	Gly Gln Pro
1085	1090	1095
Thr Leu Val Val Met Glu Leu	Met Ala His Gly Asp	Leu Lys Ser
1100	1105	1110
Tyr Leu Arg Ser Leu Arg Pro	Glu Ala Glu Asn Asn	Pro Gly Arg
1115	1120	1125
Pro Pro Pro Thr Leu Gln Glu	Met Ile Gln Met Ala	Ala Glu Ile
1130	1135	1140
Ala Asp Gly Met Ala Tyr Leu	Asn Ala Lys Lys Phe	Val His Arg
1145	1150	1155
Asp Leu Ala Ala Arg Asn Cys	Met Val Ala His Asp	Phe Thr Val
1160	1165	1170
Lys Ile Gly Asp Phe Gly Met	Thr Arg Asp Ile Tyr	Glu Thr Asp
1175	1180	1185
Tyr Tyr Arg Lys Gly Gly Lys	Gly Leu Leu Pro Val	Arg Trp Met
1190	1195	1200
Ala Pro Glu Ser Leu Lys Asp	Gly Val Phe Thr Thr	Ser Ser Asp
1205	1210	1215
Met Trp Ser Phe Gly Val Val	Leu Trp Glu Ile Thr	Ser Leu Ala
1220	1225	1230
Glu Gln Pro Tyr Gln Gly Leu	Ser Asn Glu Gln Val	Leu Lys Phe
1235	1240	1245
Val Met Asp Gly Gly Tyr Leu	Asp Gln Pro Asp Asn	Cys Pro Glu
1250	1255	1260
Arg Val Thr Asp Leu Met Arg	Met Cys Trp Gln Phe	Asn Pro Lys
1265	1270	1275
Met Arg Pro Thr Phe Leu Glu	Ile Val Asn Leu Leu	Lys Asp Asp
1280	1285	1290
Leu His Pro Ser Phe Pro Glu	Val Ser Phe Phe His	Ser Glu Glu
1295	1300	1305
Asn Lys Ala Pro Glu Ser Glu	Glu Leu Glu Met Glu	Phe Glu Asp
1310	1315	1320
Met Glu Asn Val Pro Leu Asp	Arg Ser Ser His Cys	Gln Arg Glu
1325	1330	1335
Glu Ala Gly Gly Arg Asp Gly	Gly Ser Ser Leu Gly	Phe Lys Arg
1340	1345	1350
Ser Tyr Glu Glu His Ile Pro	Tyr Thr His Met Asn	Gly Gly Lys
1355	1360	1365
Lys Asn Gly Arg Ile Leu Thr	Leu Pro Arg Ser Asn	Pro Ser
1370	1375	1380

<210> SEQ ID NO 34

<211> LENGTH: 477

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

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ggctgaagct gccctcgagg acctggtctc caccattcga gtctgaagat tctcagaagc	60
acaaccagag tgagtatgag gattcggccg gcgaatgctg ctctgtcca aagacagact	120
ctcagatcct gaaggagctg gaggagtctc cgtttaggaa gacgtttgag gattacctgc	180
acaacgtggt ttctgtccc aggccatctc ggaaacgcag gtcccttggc gatgttgga	240
atgtgacggt ggccgtgccc acggtggcag ctttcccaa cacttctcg accagcgtgc	300
ccacgagtcc ggaggagcac aggccttttg agaagggtgt gaacaaggag tcgctggtca	360
tctccggtt gcgacacttc acgggctatc gcctcgagct gcaggcttgc aaccaggaca	420
cccctgagga acggtgcagt gtggcagcct acgtcagtgc gaggaccatg cctgaag	477

<210> SEQ ID NO 35

<211> LENGTH: 513

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

ggctgaagct gccctcgagg acctggtctc caccattcga gtctgaagat tctcagaagc	60
acaaccagag tgagtatgag gattcggccg gcgaatgctg ctctgtcca aagacagact	120
ctcagatcct gaaggagctg gaggagtctc cgtttaggaa gacgtttgag gattacctgc	180
acaacgtggt ttctgtccc agaaaaacct cttcaggcac tggcgccgag gaccctaggc	240
catctcgaa acgcaggtcc cttggcgatg ttgggaatgt gacggtggcc gtgcccacgg	300
tggcagcttt ccccaacct tctcgacca gcgtgccac gagtcggag gagcacaggc	360
cttttgagaa ggtggtgaac aaggagtcgc tggtcatctc cggcttgca cacttcacgg	420
gctatcgcat cgagctgcag gcttgcaacc aggacacccc tgaggaaacgg tgcagtgtgg	480
cagcctacgt cagtgcgagg accatgacct aag	513

<210> SEQ ID NO 36

<400> SEQUENCE: 36

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<210> SEQ ID NO 37

<400> SEQUENCE: 37

000

<210> SEQ ID NO 38

<400> SEQUENCE: 38

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<210> SEQ ID NO 39

<400> SEQUENCE: 39

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<210> SEQ ID NO 40

<400> SEQUENCE: 40

000

<210> SEQ ID NO 41

-continued

<400> SEQUENCE: 41

000

<210> SEQ ID NO 42

<400> SEQUENCE: 42

000

<210> SEQ ID NO 43

<400> SEQUENCE: 43

000

<210> SEQ ID NO 44

<211> LENGTH: 119

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Ala
115

<210> SEQ ID NO 45

<211> LENGTH: 121

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln
100 105 110

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Gly Thr Thr Val Thr Val Ser Ser Ala
115 120

<210> SEQ ID NO 46
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
20 25 30
Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Ser Ser Trp Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu
100 105 110
Val Thr Val Ser Ser Ala
115

<210> SEQ ID NO 47
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Arg Ser Ser
20 25 30
Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Gly Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Gln Arg Gly His Ser Ser Gly Trp Trp Tyr Phe Asp Leu
100 105 110
Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala
115 120

<210> SEQ ID NO 48
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

-continued

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Asn Ser Ser
 20 25 30

Ser Asn Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Ala
 35 40 45

Trp Ile Gly Gly Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60

Leu Arg Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Gln Arg Gly His Ser Ser Gly Trp Trp Tyr Phe Asp Leu
 100 105 110

Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala
 115 120

<210> SEQ ID NO 49
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
 20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
 50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
 65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg Ile Thr Gly Thr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val
 100 105 110

Thr Val Ser Ser Ala
 115

<210> SEQ ID NO 50
 <211> LENGTH: 120
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
 20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Phe Phe Tyr Ser Gly Tyr Thr Asn Tyr Asn Pro Ser Leu Lys
 50 55 60

Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
 65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

-continued

Cys Ile Thr Gly Thr Thr Lys Gly Gly Met Asp Val Trp Gly Gln Gly
100 105 110

Ala Thr Val Thr Val Ser Ser Ala
115 120

<210> SEQ ID NO 51
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Phe Phe Tyr Ser Gly Tyr Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Cys Ile Thr Gly Thr Thr Lys Gly Gly Met Asp Val Trp Gly Gln Gly
100 105 110

Thr Thr Val Thr Val Ser Ser Ala
115 120

<210> SEQ ID NO 52
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 52

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
20 25 30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65 70 75 80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
85 90 95

Ser Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

<210> SEQ ID NO 53
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Glu Asn Asn

20								25					30				
His	Val	Ser	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu		
		35					40					45					
Ile	Tyr	Asp	Asn	Asn	Lys	Arg	Pro	Ser	Gly	Ile	Pro	Asp	Arg	Phe	Ser		
		50					55					60					
Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Thr	Leu	Gly	Ile	Thr	Gly	Leu	Gln		
		65					70					75			80		
Thr	Gly	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Glu	Thr	Trp	Asp	Thr	Ser	Leu		
				85					90					95			
Ser	Ala	Gly	Arg	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Gly		
				100					105					110			

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<210> SEQ ID NO 54
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 54

Asp 1	Ile	Gln	Met 5	Thr	Gln	Ser	Pro	Ser	Ser	Val 10	Ser	Ala	Ser	Val 15	Gly
Asp	Arg	Val	Thr 20	Ile	Thr	Cys	Arg	Ala 25	Ser	Gln	Gly	Ile	Ser 30	Ser	Trp
Leu	Ala	Trp 35	Tyr	Gln	Gln	Lys	Pro 40	Gly	Lys	Ala	Pro	Lys 45	Leu	Leu	Ile
Tyr	Ala 50	Ala	Ser	Ser	Leu	Gln 55	Ser	Gly	Val	Pro	Ser 60	Arg	Phe	Ser	Gly
Ser 65	Gly	Ser	Gly	Thr	Asp 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Leu	Gln	Pro 80
Glu	Asp	Phe	Ala	Thr 85	Tyr	Tyr	Cys	Gln	Gln	Ala	Asn	Ser	Phe	Pro 95	Phe
Thr	Phe	Gly 100	Pro	Gly	Thr	Lys	Val	Asp 105	Ile	Lys	Arg				

```
<210> SEQ ID NO 55
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 55

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Val	Ser	Ala	Ser	Val	Gly
1				5						10				15	
Asp	Ser	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Gly	Ile	Ser	Ser	Tyr
			20					25					30		
Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile
		35					40					45			
Tyr	Ala	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				
Asn	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70					75					80
Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ala	Asn	Asn	Phe	Pro	Phe
				85					90					95	
Thr	Phe	Gly	Pro	Gly	Thr	Lys	Val	Asp	Ile	Lys	Arg				
			100					105							

```
<210> SEQ ID NO 56
<211> LENGTH: 108
<212> TYPE: PRT
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-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Arg Gly Ile Ser Ser Trp
 20 25 30

Leu Ala Trp Tyr Gln Gln Arg Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Thr Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Phe
 85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
 100 105

<210> SEQ ID NO 57

<211> LENGTH: 112

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
 65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
 85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

<210> SEQ ID NO 58

<211> LENGTH: 112

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

Gln Ser Val Leu Thr Gln Ala Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Arg Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Phe Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
 65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
 85 90 95

-continued

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

<210> SEQ ID NO 59
 <211> LENGTH: 112
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Gly Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
 65 70 75 80

Gln Ala Asp Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser
 85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

What is claimed is:

1. A method for detecting or quantifying an insulin receptor isoform A (IR-A) nucleic acid in a biological sample or a sample of nucleic acids prepared from a biological sample, comprising the steps of:

- (i) contacting a biological sample or nucleic acids prepared from a biological sample with a primer set under conditions suitable for polymerase-based amplification;
- (ii) amplifying the IR-A nucleic acid by performing polymerase-based amplification; and
- (iii) detecting or quantifying the amplified IR-A nucleic acid using a kit;

wherein the kit is selected from:

- (a) a kit comprising a 20 nucleotide IR-A forward synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 3, a 20 nucleotide IR-A reverse synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 21, and
- a 14 nucleotide synthetic nucleic acid IR-A probe of a nucleotide sequence set forth in SEQ ID NO: 7, wherein the probe comprises a detectable label; or
- (b) a kit comprising a 22 nucleotide IR-A forward synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 5, an 18 nucleotide IR-A reverse synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 22, and
- a 17 nucleotide synthetic nucleic acid IR-A probe of a nucleotide sequence as set forth in SEQ ID NO: 8 wherein the probe comprises a detectable label.

2. The method of claim 1, wherein the biological sample or nucleic acids prepared from a biological sample is contacted with a 20 nucleotide IR-A forward synthetic nucleic acid primer of the nucleotide sequence as set forth in SEQ ID NO: 3,

- a 20 nucleotide IR-A reverse synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 21, and

a 14 nucleotide synthetic nucleic acid probe of the nucleotide sequence as set forth in SEQ ID NO: 7 comprising a detectable label.

3. The method of claim 1, wherein the biological sample or nucleic acids prepared from a biological sample is contacted with

- a 22 nucleotide IR-A forward synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 5,
- an 18 nucleotide IR-A reverse synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 22, and
- a 17 nucleotide synthetic nucleic acid probe of a nucleotide sequence as set forth in SEQ ID NO: 8 comprising a detectable label.

4. A method for detecting or quantifying an Insulin Receptor Isoform B (IR-B) nucleic acid in a biological sample or in a sample of nucleic acids prepared from a biological sample comprising the steps of:

- (i) contacting the biological sample, or the nucleic acids prepared from a biological sample with a primer set under conditions suitable for polymerase-based amplification;
- (ii) amplifying the IR-B nucleic acid by performing polymerase-based amplification; and
- (iii) detecting or quantifying the amplified IR-B nucleic acid using a kit; wherein the kit comprises: a primer set of a 21 nucleotide IR-B forward synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 11; a 19 nucleotide IR-B reverse synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 12; and a 15 nucleotide synthetic nucleic acid IR-B probe, wherein the IR-B probe comprises a detectable label, and has a nucleotide sequence as set forth in SEQ ID NO: 13; SEQ ID NO: 14; or SEQ ID NO: 15.

5. The method of claim 1, wherein the detectable label is a fluorescent label.

6. The method of claim 2, wherein the detectable label is a fluorescent label.

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7. The method of claim 3, wherein the detectable label is a fluorescent label.

8. The method of claim 4, wherein the detectable label is a fluorescent label.

9. A method for determining the IR-A:IR-B ratio in a biological sample or a sample of nucleic acids prepared from a biological sample, comprising
 quantifying IR-A by using the method of claim 1; and
 quantifying IR-B by using an IR-B forward synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 11, an IR-B reverse synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 12, and a 15 nucleotide synthetic nucleic acid IR-B probe, wherein the IR-B probe comprises a detectable label, and has a nucleotide sequence as set forth in SEQ ID NO: 13; SEQ ID NO: 14; or SEQ ID NO: 15; and determining the IR-A to IR-B ratio.

10. The method of claim 9, wherein the detectable label is a fluorescent label.

11. The method for determining the ratio of IR-A to IR-B of claim 9, wherein the method comprises quantifying IR-A by using

an IR-A forward synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 3, a 20 nucleotide IR-A reverse synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 21, and a 14 nucleotide synthetic nucleic acid IR-A probe of a nucleotide sequence as set forth in SEQ ID NO: 7, wherein the probe comprises a detectable label; quantifying IR-B by

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using an IR-B forward synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 11, an IR-B reverse synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 12, and a 15 nucleotide synthetic nucleic acid IR-B probe, wherein the IR-B probe comprises a detectable label, and has a nucleotide sequence as set forth in SEQ ID NO: 13; and determining the IR-A to IR-B ratio.

12. The method of claim 11, wherein the detectable labels are fluorescent labels.

13. The method for determining the ratio of IR-A to IR-B of claim 9, wherein the method comprises: quantifying IR-A by using an IR-A forward synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 5, an 18 nucleotide IR-A reverse synthetic nucleic acid of a nucleotide sequence as set forth in SEQ ID NO: 22, and a 17 nucleotide synthetic nucleic acid IR-A probe of a nucleotide sequence as set forth in SEQ ID NO: 8, wherein the probe comprises a detectable label; quantifying IR-B by using an IR-B forward synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 11, an IR-B reverse synthetic nucleic acid of the nucleotide sequence as set forth in SEQ ID NO: 12, and a 15 nucleotide synthetic nucleic acid IR-B probe, wherein the IR-B probe has a detectable label attached at one end, and has a nucleotide sequence as set forth in SEQ ID NO: 13; and determining the IR-A to IR-B ratio.

14. The method of claim 13, wherein the detectable labels are fluorescent labels.

* * * * *